EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

PUBLISHED BY

THE SOCIETY FOR THE STUDY OF EVOLUTION

Vol. XIII DECEMBER, 1959 No. 4

CANALIZATION OF THE SCUTE PHENOTYPE OF DROSOPHILA

J. M. RENDEL

CSIRO Animal Genetics Section, Zoology Department, Sydney University, N.S.W., Australia

Received November 17, 1958

INTRODUCTION

Waddington (1953) has shown that a character, cross-veinless, in *D. melanogaster,* which is virtually invariant in normal surroundings and so not susceptible to selective breeding, can be made to vary and to become susceptible to selective breeding by heat applied during the larval stage. Dun and Fraser (1958) have shown that the presence of the tabby gene in mice reveals genetic variation in whisker number, which responds to selective breeding and which was not visible in the normal mouse. There is abundant evidence that the manifestation of genetic variation in the phenotype depends on the environment and the rest of the genotype. Waddington, in a series of papers summarized in his book (1958), has suggested that where it is of advantage to an animal for its development to follow a certain path, a genotype will evolve which will resist forces tending to change the path; in his terminology the developmental path, or creode, is canalized.

Dun and Fraser suppose that the tabby gene disrupts the normal canalization. Figure 1 is taken from Dun and Fraser's diagram of the relationship between the whisker number phenotype and genotype of wild type and tabby mice. Tabby is sex-linked, so segregating families from a back-cross of heterozygotes to normals have four genotypes: tabby males, heterozygous females and homozygous normal males and females. The last two are phenotypically very constant in whisker number; the tabby male is less variable than the heterozygous female. Dun and Fraser suppose that the genetic variation of whisker number is independent of the tabby gene. The tabby gene lowers the mean and disrupts the system, whatever it may be, which canalizes the development of whisker number. Disruption of canalization leads to the three separate dose-response curves shown in the diagram. These curves are supposed to show the change of phenotype for a given change of genotype in the three types of mice. When high whisker number is selected for in tabby genotypes, the genotype for whisker number is raised; and, eventually, as they have shown, the whisker number of wild type is also increased. Response to selection should follow three separate dose-response curves

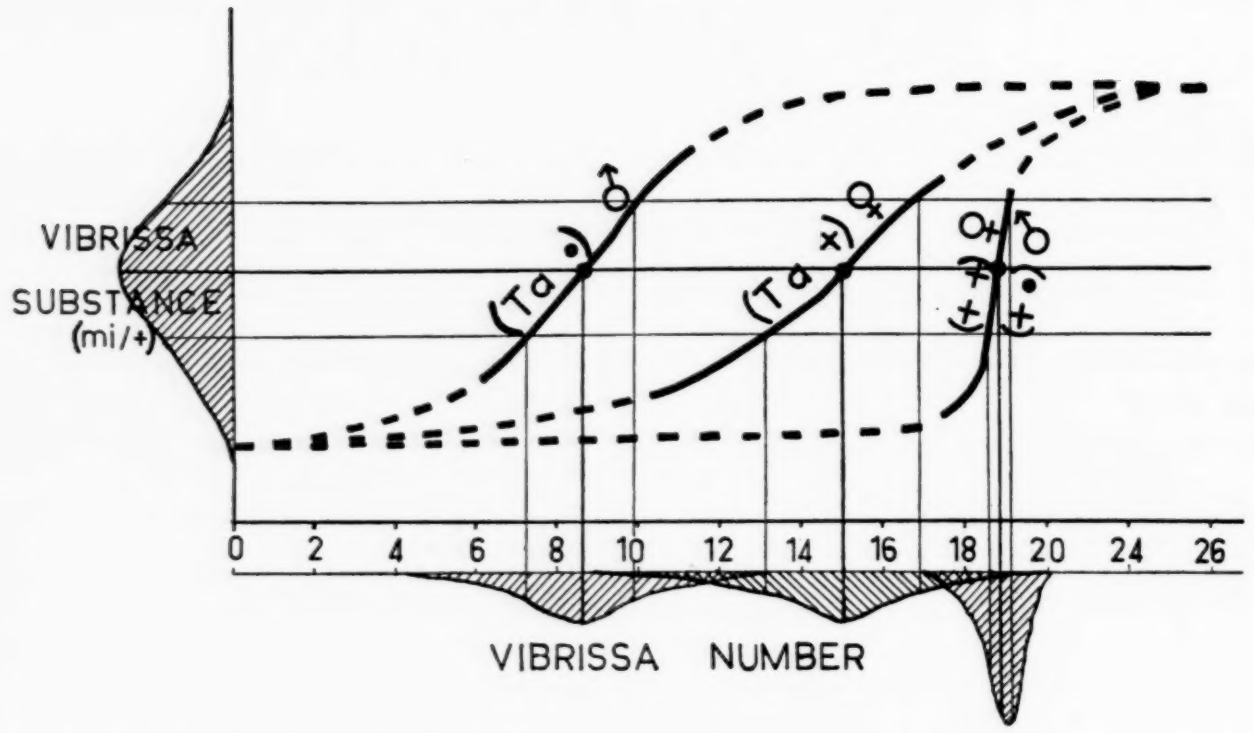


FIG. 1. Relationship between variation in genotype and phenotype in tabby and wild type mice. Axis represents phenotype, abscissa genotype (after Dun and Fraser).

in the three genotypes and all genotypes should become more variable. An alternative hypothesis is that there is a single gene dose-response curve; that the course of development is canalized; and that the degree of expression of the character is what determines the degree of canalization. The difference between the two hypotheses is small; under that of Dun and Fraser, one would expect selection to cause all genotypes to become more variable; the alternative leads one to expect that as the mutant phenotype approaches the wild type it will become less variable, whilst sibs with phenotypes surpassing the wild type will increase in variability. The experiment described here supports the latter hypothesis.

EXPERIMENTAL PROCEDURE

A stock of flies in which the female parents are $y + w^{w}/+ sc\ w^{bl}$ and the male parents $sc\ w^{bl}$ segregates to produce $y\ w^{w}$ and $sc\ w^{bl}$ males with only occasional recombinants $y + w^{bl}$ and $sc\ w^{w}$; the female segregants are $y + w^{w}/+ sc\ w^{bl}$ and $+ sc\ w^{bl}/+ sc\ w^{bl}$ with occasional recombination classes $+ sc\ w^{bl}/+ sc\ w^{w}$ and $+ sc\ w^{bl}/y + w^{bl}$. Very occasionally recombination takes place between *y* and *sc*, producing a $y\ sc\ w^{bl}$ and $+ + w^{w}$ chromosome. The stock is kept going by mating $+ sc\ w^{bl}$ males to $+ sc\ w^{bl}/y + w^{w}$ females which can be recognized by eye color, since w^{bl}/w^{w} is distinguishable from both w^{w}/w^{w} and w^{bl}/w^{bl}; and by scute, *sc* being recessive to +. If recombination has occurred between *y* and *sc*, a female $+ + w^{w}/+ sc\ w^{bl}$ will be detected by her progeny. Recombination between *sc* and *y* is expected on the order of 1 in 1,500 times, and so far no female carrying a $+ + w^{w}$ chromosome has been used as a parent. It is possible to mistake a *sc*/*sc* female with four scutellar bristles for a wild type *sc*/+ female if the scutellar bristles only are taken into account; but *sc*/*sc* flies have about half as many abdominal chaetae as *sc*/+ flies, so that no error is made when both criteria are used.

A stock of $+ sc\ w^{bl}/y + w^{w}$ and $+ sc\ w^{bl}$ was subdivided into lines, and in each line male parents were selected for increase in scutellar bristle number. The five males with the most scutellar bristles were mated to five heterozygous females in each generation. After 10 generations

sublines homozygous for *sc* w^{bl} were taken off, and in these lines parents of both sexes were chosen on their scutellar bristle number. No details of selection differential, rate of response to selection, or heritability have been extracted from this experiment because several parents were used in each generation and it is not possible to say how much each contributed to the next generation. It is true that in later generations this does not matter because all parents had four scutellar bristles; but there is the further objection that virginity of female parents cannot be guaranteed, and so the male parent is not certainly known. The incidence of non-virginity was not very high, but some *yw*/*yw* females were found showing that it did occur. A *yw*/*yw* female with five scutellar bristles which appeared in one line was used to start a stock in which parents of both sexes were selected for their scutellar bristle number; in the other selection lines no females with more than four bristles have been bred from. In the heterozygous selection lines young heterozygous females with four bristles were taken at random, those with five being rejected in case their extra bristle was due to something other than the selection in males; as they become more common this precaution can be dropped; in the homozygous lines no female with more than four bristles has yet appeared.

As the bristle number increased in the selection lines, occurrence of two bristles very close together became more frequent; flies with four bristles or fewer occasionally had the bristles placed so that two were close together at one of the normal bristle sites, whereas other normal sites were empty; but in no case has a fly with five or more bristles been seen with any of the four normal sites vacant. Usually a fly with five or more bristles carried the extra bristles near a normal bristle site; but this was not always so and there seems no sharp demarcation between flies in which five bristles were evenly spaced, and those in which two of the five appeared to share a site (see fig. 2). All bristles have been scored no matter where they were placed; but in the main selection lines only flies with each bristle at a separate site have been bred from. The suspicion I had at one stage, that the possession of two bristles at one site might be a different phenomenon from the general increase in bristle number, was not supported by the increase in incidence of such bristles, despite the fact that only flies with one bristle at a site were selected for breeding. In the *y* w^{w}/*y* w^{w} high bristle number line all flies with more than four bristles were acceptable no matter how the bristles were placed. The bristles referred to here were all separate bristles and are not to be confused with split or double bristles.

In order to maintain a brisk response to selection the heterozygous lines were intercrossed after the 10th generation and ten fresh lines extracted from the cross. These lines were maintained until the 15th generation, when one was lost; after

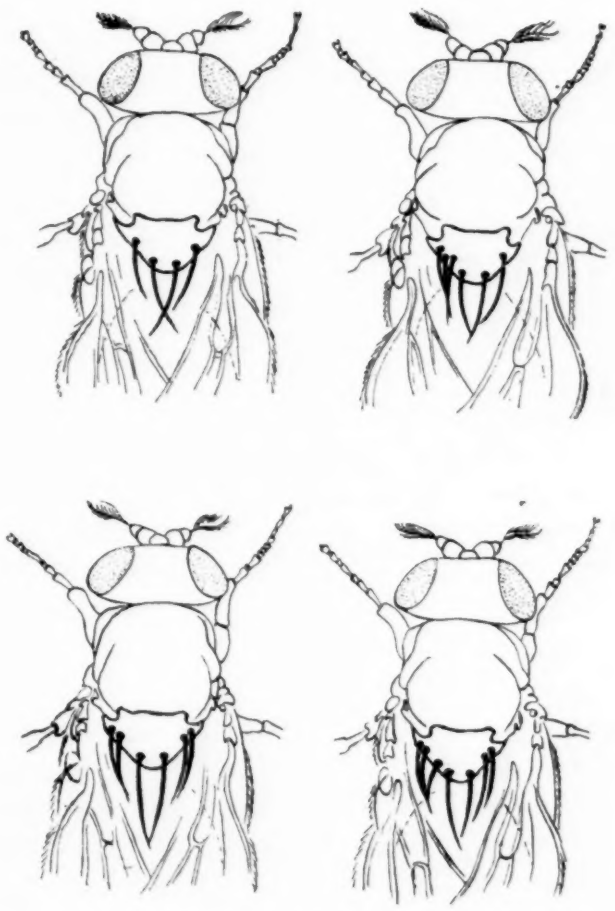


FIG. 2. Distribution of bristles in some typical flies of the *yw* selection line.

TABLE 1. *Average number of bristles on the scutellum and the average number of chaetae on the last segment of the first twenty flies of each sex to hatch in the first generation of ten heterozygous selection lines*

	sc ♂♂		+ ♂♂		$\frac{sc}{+}$ ♀♀	
Line	Scutellar	Abdominal	Scutellar	Abdominal	Scutellar	Abdominal
1	1.1	6.9	4	21.4	4	22.1
2	0.8	7.2	4	18.2	4	19.6
3	0.8	7.2	4	20.1	4	19.6
4	1.0	7.3	4	19.3	4	21.6
5	1.2	8.1	4	20.2	4	22.9
6	1.3	6.8	4	21.2	4	21.0
7	0.8	6.5	4	19.5	4	21.7
8	0.6	7.2	4	19.5	4	23.3
9	1.1	7.0	4	18.9	4	20.5
10	0.7	6.9	4	19.0	4	19.5

the 17th the lines were again intercrossed and cut back to 5 until the end of the experiment. Ten lines were kept in the homozygous selection experiment for the first nine generations when one was lost through contamination; at the 13th generation the lines were intercrossed and reduced to five until the end of the experiment.

The number of abdominal chaetae, which was used to distinguish *sc/sc* females with four bristles, tended in general to decline slightly in all genotypes as the number of scutellar bristles increased; the number of chaetae on the last segment of *sc/+* flies fell to 12.1 in one line, but as the number in *sc/sc* of the same line fell to 6.2 it was still possible to tell *sc/+* from *sc/sc*. Table 1 shows the number of bristles and chaetae in the ten lines at the start of selection; table 2 shows the number in eight of the nine lines at the 15th generation. The first generation were offspring of + + +/+ *sc* w^{bl} ♀♀ mated to *y* + w^{w} ♂♂ so the genotypes segregating were not the same as in later generations.

The results for separate lines have been summed and analyzed together. The justification for the summation comes from analysis of variance of lines in the second and eighth generation. Although differences between lines are significant they contribute only a small fraction to the score of individual flies.

RESULTS AND INTERPRETATION

The result of selecting male parents for their scutellar bristle number in the heterozygous lines is shown graphically in

TABLE 2. *Average number of bristles on the scutellum and average number of chaetae on the last segment of the first ten flies of each sex to emerge in the 15th generation of eight heterozygous selection lines*

	sc ♂♂		*yw* ♂♂		$\frac{sc}{+}$ ♀♀		$\frac{sc}{sc}$ ♀♀	
Line	Scutellar	Abdominal	Scutellar	Abdominal	Scutellar	Abdominal	Scutellar	Abdominal
1	1.6	6.8	4	19.0	4	19.7	2.3	9.7
2	1.9	4.9	4	14.7	4	12.1	2.4	6.2
3	2.6	7.7	4	17.2	4	19.0	3.0	9.0
4	2.5	7.1	4	19.2	4	18.6	2.8	9.4
5	2.0	6.5	4	17.1	4	19.8	2.8	9.4
6	2.9	5.5	4	16.5	4	16.9	3.7	8.8
7	2.2	5.7	4	17.8	4	20.1	2.8	8.5
8	1.7	6.8	4	17.3	4	20.1	2.4	9.4

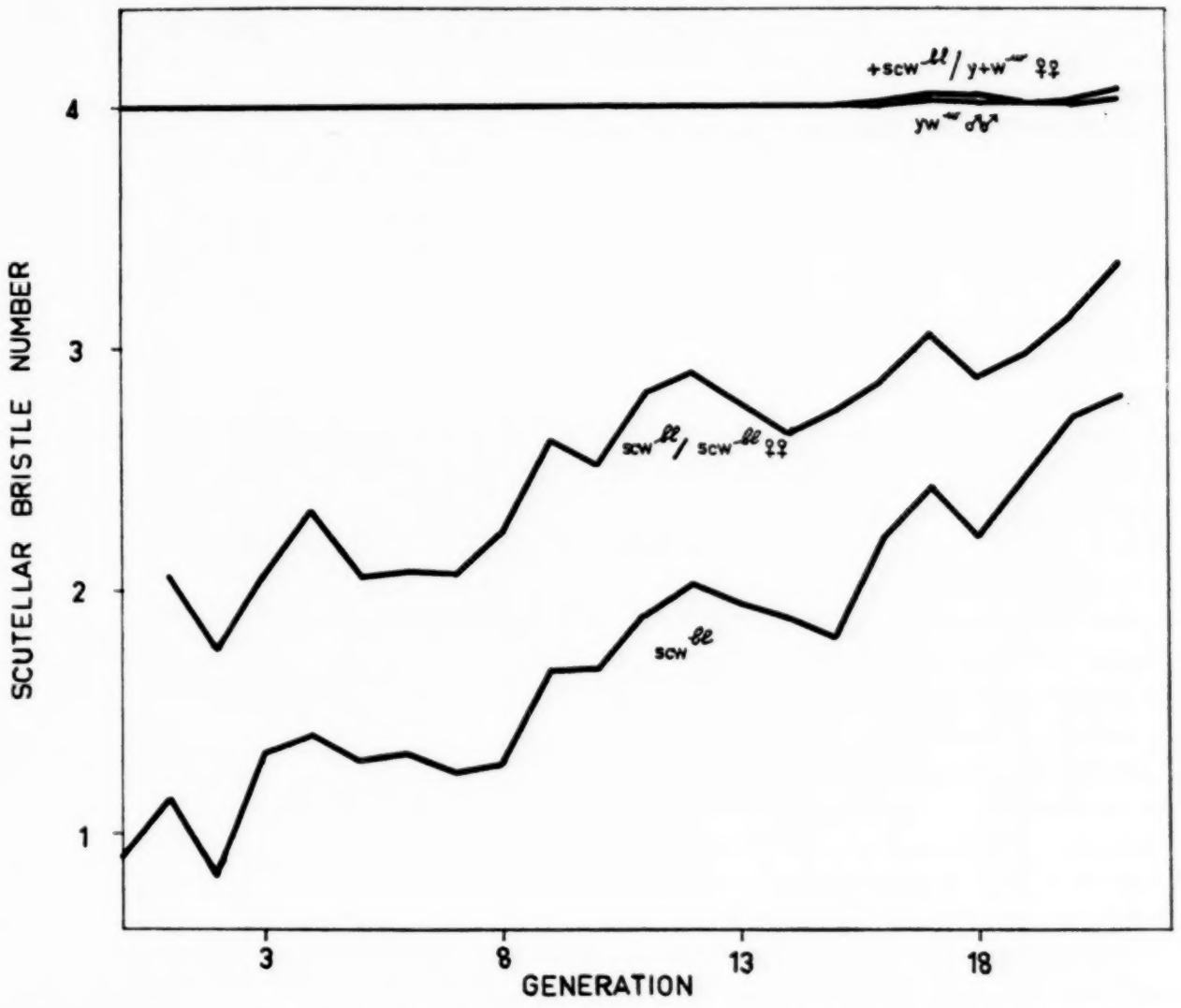


FIG. 3. Progress of selection for scutellar bristles in a population segregating for scute.

figure 3. The average number of scutellar bristles in scute males starts at about one and rises to 2.8 in the 22nd generation; in scute females it starts at just over two and finishes at 3.3. The difference between males and females diminishes steadily as bristle number increases; the variance of the number diminishes in females once bristle numbers begin to pile up against the 4-bristle class; in males there is little change throughout; this is shown in table 3, where the actual scores of the flies is set out for each generation, and in table 4, which lists the variance of each generation. No scute fly was found with more than four scutellar bristles despite the increasing number with four and this accounts for the drop in variance in females as well as the fall in difference between males and females; as the mean number in females increases, more and more flies reach the phenotypic ceiling of four. In males the distribution is cut off at zero at the start of the experiment and becomes approximately normal by the 9th to 14th generation; so the rate of increase slows down in females but not in males and the two approach one another. If no flies with five bristles appear, we may expect the two sexes to become equal when all scute flies of both sexes have four bristles. The *yw* males and *sc*/+ ♀♀ segregating at the same time stay consistently at four bristles for the first fifteen generations; very seldom a fly with three or one with five bristles appears, but such exceptions are rare. At the 15th generation flies with five bristles begin to appear more regularly and later, flies with six; the genotype which was responsible for raising the bristle number in scute flies has made itself felt in the end in + and *sc*/+ flies, but not until a genetic change has been built up capable

TABLE 3. *Progress of selection for scutellar bristles in the heterozygous selection lines segregating for* sc, y *and* w

Generation	*yw* ♂♂ No. of bristles 3	4	5	6	Mean	*sc w^{bl}* ♂♂ No. of bristles 0	1	2	3	4	Mean	$\frac{sc\ w^{bl}}{sc\ w^{bl}}$ ♀♀ No. of bristles 0	1	2	3	4	Mean	$\frac{sc\ w^{bl}}{yw}$ ♀♀ No. of bristles 3	4	5	6	Mean
1		199			4.00	69	77	44	5		.92								(200)			
2	1	179			4.0−	51	66	62	8		1.14	8	22	73	33	8	2.076		175			4.00
3		148			4.00	69	74	37	1		.83	8	25	59	10	3	1.76		101			4.00
4		199	1		4.0+	35	83	77	14		1.33	5	40	94	40	12	2.07		200			4.00
5		306			4.00	56	120	135	25	2	1.40	10	34	164	92	32	2.31		319			4.00
6		202			4.00	43	65	86	10		1.31	11	43	94	46	16	2.06		202			4.00
7		440			4.00	98	154	184	26	3	1.32	6	71	277	94	15	2.09	1	369	2		4.0+
8		249	1		4.0+	65	81	113	8	1	1.25	8	46	129	55	12	2.07		297			4.00
9		230			4.00	52	65	75	17		1.27	4	24	89	40	19	2.26		224			4.00
10		280			4.00	33	72	138	40	2	1.67	2	8	100	69	40	2.63	1	323			4.0−
11		171	1		4.0+	16	52	78	25	2	1.68	0	8	33	21	13	2.52		150	1		4.0+
12	1	270			4.0−	16	43	127	45	4	1.91	2	7	54	64	42	2.81		252			4.00
13		286			4.00	11	38	138	44	13	2.04	1	4	74	86	65	2.91		223			4.00
14		274	1		4.0+	21	53	124	56	12	1.94	0	3	77	67	43	2.79		307	1		4.0+
15		244	1		4.0+	15	49	138	40	6	1.89	2	7	80	68	35	2.66		195			4.00
16	1	409			4.0−	34	87	220	53	14	1.82	4	9	137	121	78	2.74		312	1		4.0+
17		373	12		4.034	11	36	163	79	26	2.23	0	2	105	97	77	2.89		375	3		4.01
18		348	16	1	4.049	2	21	206	148	28	2.44	0	4	105	151	127	3.04		240	4		4.02
19		435	13	2	4.038	5	54	276	141	21	2.24	0	0	123	166	83	2.89		395	6		4.02
20	1	294	1		4.00	5	25	115	76	44	2.49	0	1	73	72	64	2.95		251			4.00
21		165	3		4.018	0	3	73	87	70	2.96	0	0	37	43	56	3.14		170	2		4.01
22		188	12		4.060	0	10	59	77	43	2.81	0	0	29	56	90	3.35		135	3		4.02

TABLE 4. *Variances of bristle number in* sc *males and females of the heterozygous selection lines*

Generation	*sc w^bl* ♂♂ No.	*sc w^bl* ♂♂ Mean square	$\frac{sc\ w^{bl}}{sc\ w^{bl}}$ ♀♀ No.	$\frac{sc\ w^{bl}}{sc\ w^{bl}}$ ♀♀ Mean square
1	195	0.68	—	—
2	187	0.76	144	0.82
3	181	0.58	105	0.70
4	209	0.69	191	0.77
5	338	0.75	332	0.79
6	204	0.73	210	0.94
7	465	0.78	463	0.53
8	268	0.75	250	0.72
9	209	0.86	176	0.82
10	285	0.78	219	0.72
11	173	0.76	75	0.81
12	235	0.70	169	0.80
13	244	0.73	230	0.71
14	266	0.91	190	0.65
15	248	0.69	192	0.73
16	408	0.78	349	0.48
17	315	0.78	281	0.66
18	405	0.52	387	0.64
19	497	0.54	372	0.55
20	265	0.89	210	0.66
21	233	0.67	136	0.66
22	189	0.71	175	0.56

of shifting the mean bristle number of scute flies nearly two bristles.

The plot in figure 2 shows only means. It is instructive to make a plot showing the distribution about the means; so the figures in table 3 have been converted into percentages and expressed in probits in table 5. The method of calculating them can best be described by taking an example. The *sc w^bl* ♂♂ of generation 10 were distributed as follows; 1.58% had no scutellar bristle, 36.84% had 0 or 1, 85.26% had 0, 1 or 2, and 99.30% had 0, 1, 2 or 3; 2 flies or 0.7% had 4 bristles. In this generation one fly corresponds to 0.35% of the population and has a probit value of ±2.7. I assume therefore that the flies counted scan a probit distance from −2.7 to +2.7 and the starting point is taken as −2.7. 11.58% corresponds to a probit value of −1.2, so that flies with no scutellar bristles stretch from −1.2 to at least −2.7, i.e. cover at least 1.5 probit units, but as there is no way of telling how far the 0 scale would stretch, this is an incomplete class. 36.84% corresponds to a probit score of −0.3 so that in this generation flies with one bristle occupy a place on the scale from −1.2 to −0.3 or 0.9 probits; similarly, the flies with two bristles stretch from −0.3 to +1 and those with three from +1 to +2.4; those with four are an incomplete class stretching for at least 0.3 probits beyond the 3-bristle class. If the phenotypic classes reflect different levels of an underlying variable, whose distribution does not change markedly from generation to generation, the probit distance between class and class is a relative measure of difference in dosage of the variable. Although in any one generation variation about the mean will include some environmental variation, differences between generations, barring random fluctuations, can be regarded as genetic. No matter what percentage the class is of the population, so long as the variable behind it is reasonably constant in distribution it should occupy the same probit distance.

The concept is of a variable, distributed about a mean value which is being pushed by selection up a genotypic scale. This scale has, corresponding to it, a phenotypic scale but the correspondence is far from exact. Thus a shift of mean which has taken *sc* males from 0.92 to 2.81 bristles has shifted *yw* males from 4.00 to 4.06; it has taken *sc*/*sc* females from 2.08 to 3.35 and barely shifted *sc*/+ females. Further the probit distance subtending the 1-bristle class is on average about 0.9 whereas the distance subtending the 2-bristle class is on average about 1.5. Had this difference been one inherent in the distribution of the underlying variable one would expect it to be transferred from class to class as the mean moved up the scale. But the unevenness is associated with the bristle class and one must suppose that different dosages of the variable are required to move from class to class; these dosages are roughly indicated by the probit distance subtending the class.

It is simple to estimate a relative score

TABLE 5. *Probit distance occupied by bristle classes of flies of the heterozygous selection lines segregating for* sc, y *and* w

Generation	*sc w^{bl}* ♂♂ No. of bristles					$\frac{sc\ w^{bl}}{sc\ w^{bl}}$ ♀♀ No. of bristles					*yw* ♂♂ No. of bristles			$\frac{sc\ w^{bl}}{yw}$ ♀♀ No. of bristles	
	0	1	2	3	4	0	1	2	3	4	4	5	6	4	5
1	2.19*	1.01	1.27	0.63*		0.87*	0.78	1.38	1.03	0.87*	±2.58*				
2	1.94*	0.96	1.37	0.83*		0.92*	0.95	1.64	0.74	0.45*	±2.54*			±2.53	
3	2.24*	1.11	1.73	—		0.62*	1.22	1.33	0.92	1.03*	±2.48*			±2.33	
4	1.62*	1.13	1.34	1.09*		0.87*	0.77	1.43	0.98	1.45*	±2.58*			±2.58	
5	1.78*	1.02	1.36	1.11	0.23*	0.96*	0.98	1.19	0.89	1.16*	±2.69*			±2.74	
6	1.78*	0.87	1.58	0.93*		0.62*	1.26	1.69	1.13	1.00*	±2.58*			±2.58	
7	2.06*	0.91	1.43	0.94	0.38*	0.80*	1.06	1.41	1.04	0.99*	±2.85*			5.12	0.23*
8	1.98*	0.81	1.71	0.86		0.53*	1.00	1.43	0.81	1.29*	±2.65*			±2.71	
9	1.99*	0.83	1.25	1.27*		0.24*	0.68	1.68	0.90	1.50*	±2.63*			±2.61	
10	1.50*	0.86	1.39	1.41	0.24*	—	0.98*	1.36	0.82	1.29*	±2.52*			±2.74	
11	1.20*	1.05	1.28	1.16	0.25*	0.26*	0.64	1.30	1.00	1.84*	±2.69*			±2.48	
12	1.14*	0.82	1.48	1.36	0.46*	—	0.62	1.61	0.97	2.06*	±2.52*			±2.65	
13	0.94*	0.86	1.57	0.89	1.02*	—	0.42*	1.94	0.94	1.81*	±2.68*			±2.61	
14	1.26*	0.82	1.25	1.04	0.97*	0.23*	0.67	1.58	1.00	1.65*	±2.70			±2.72	
15	1.10*	0.90	1.55	1.08	0.67*	0.50*	0.47	1.61	0.94	2.00*	±2.64*			±2.58	
16	1.42*	0.86	1.51	0.84	0.99*	—	0.24*	2.15	0.90	2.09*	±2.82*			±2.75	
17	0.94*	0.77	1.47	0.96	1.36*	—	0.46*	1.75	1.03	2.34*	4.65*	0.95*		4.82*	0.38*
18	0.23*	1.00	1.74	1.32	1.33*	—	—	2.36*	1.09	2.11*	4.59*	0.97		4.28*	0.51*
19	0.55*	1.15	1.63	1.28	1.15*	—	—	1.98	0.89	2.03*	4.75*	0.72	0.23*	4.36*	0.63*
20	0.60*	0.86	1.33	0.85	1.70*	—	—	1.83*	0.83	2.22*	±2.71*			±2.65	
21	—	0.40*	1.78	0.97	2.11*	—	—	1.56*	1.01	2.49*	4.61*	0.41*		4.52	0.26*
22	—	0.94*	1.27	1.10	1.81*	—	—				3.93*	1.53*		4.08*	0.43*
Average		0.93	1.46	1.07			0.92	1.58	0.95						

* Values are minima; they represent the tails of the distribution and so are incomplete classes and are not included in the average.

on the supposed genetic scale for 1-, 2- and 3-bristle classes; it is simply the mean probit score, taken from all generations in which the class was a complete class. The genetic score spanned by the 4-bristle class is not too easy to measure and the 0 class cannot be measured at all. An estimate of the 4-bristle class has been made as follows: in the present experiment, as scute males and females disappear into what may be called the 4-bristle shadow, wild type sibs have started to appear in the 5-bristle class. At the beginning of the experiment some one in 800 wild-type flies had only three bristles. This is a fraction corresponding to −3.4 probits, so the 3-bristle line can be assumed to lie 3.4 units below the population mean. In the fifteen generations of selection that intervened before 5-bristle flies started to appear regularly the mean of the scute sibs had shifted about two units, so that the mean of wild type flies when 5-bristle flies begin to appear regularly can be taken as 5.4 units from the top of the 3-bristle margin. The bottom of the 5-bristle margin at this point is about +2.7 probits from the mean, so that the total spread of the 4-bristle character is 8.1 units. This can be compared with 1 and 1.5 units of the 1-, 2- and 3-bristle classes and shows a much stronger degree of canalization. Once 5-bristle flies start appearing regularly, 6-bristle flies are quick to follow. The 5-bristle class does not occupy much more than 1.5

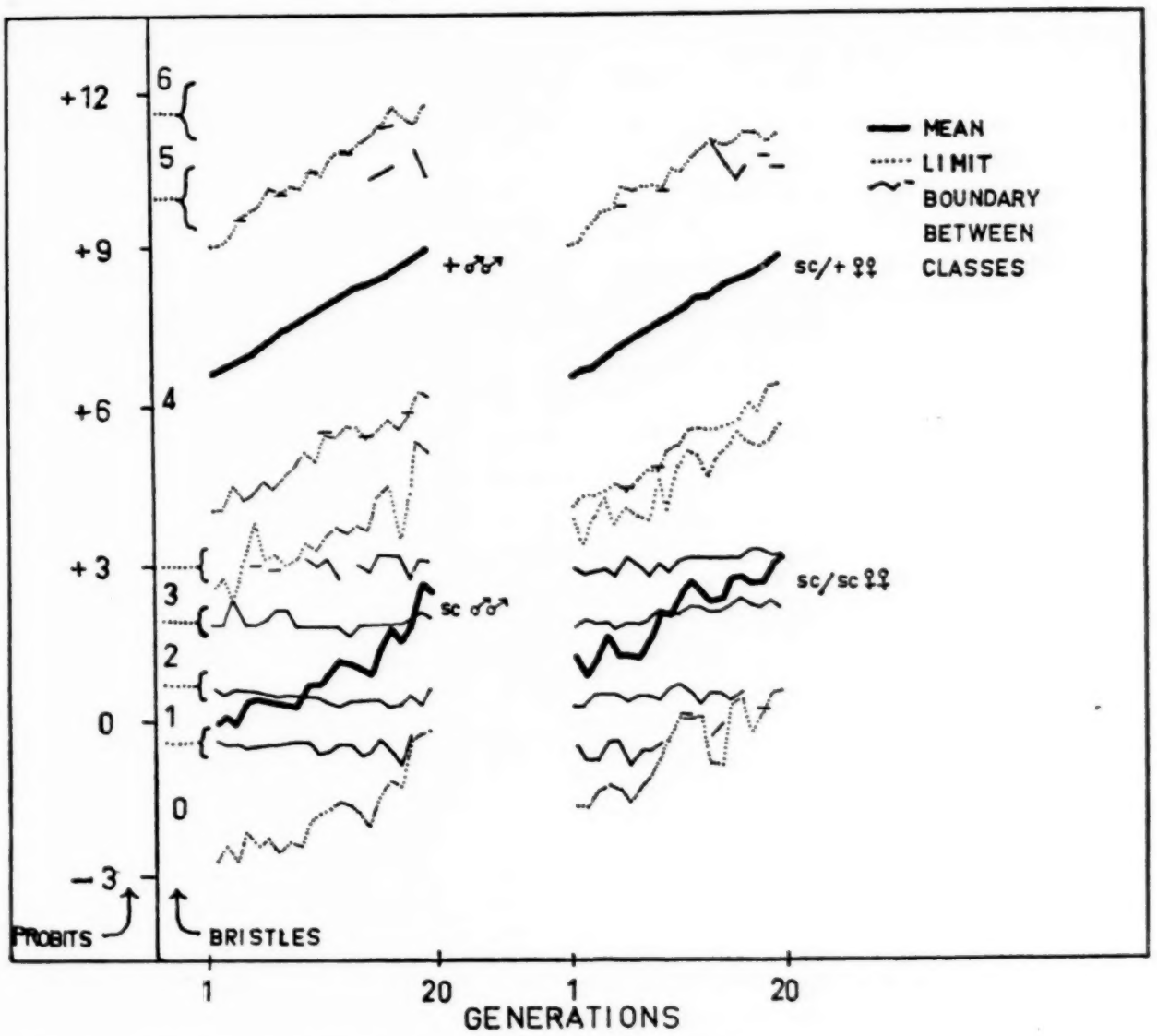


FIG. 4. Progress of selection for scutellar bristles expressed in probits. Axis represents generations; abscissa assumes genotypic value meaured in probits. Thick lines ——— represent changes in the mean, thin lines ——— boundaries between bristle classes. Dots . . . . . boundaries of incomplete classes.

TABLE 6. *Selection progress in the* yw *selection line*

Generation	♂♂ No. of bristles 3	4	5	6	Mean	♀♀ No. of bristles 3	4	5	6	7	Mean
1		99			4.00		75	3			4.04
2		100	1		4.01		87	10			4.10
3		104	1		4.01		84	20	9		4.34
4		58	4		4.06		44	29	9		4.57
5		62	11	1	4.18		53	24	5		4.41
6		35	5		4.13		44	7	1		4.17
7		68	10	3	4.20		37	22	5		4.50
8		70	13	2	4.20		23	20	9		4.73
9		54	20	1	4.30		13	26	25	1	5.21
10		68	70	12	4.63		34	65	43	1	5.08

units, in fact it takes much the same genetic change to move through five to six as it does to move through one to two, or two to three, or three to four; but eight times as great a genetic change is needed to go through four to five bristles.

The change of genotypic value from generation to generation which is supposed to have taken place is illustrated in figure 4. In this figure the dark thick line marks the mean round which the variable is distributed each generation. This mean is positioned on the figure as follows: suppose the mean falls between two and three, say 2.5; the mean then lies halfway up the 2-bristle class which occupies about 1.5 probits; so, on the genotypic scale, the mean of the distribution falls 0.75 units from the line taken as the starting point of the 2-bristle class. This is then the central point from which the rest of that generation is plotted. It is

TABLE 7. *Probit distance occupied by bristle classes of flies of the yellow white selection line*

♂♂ No. of bristles 4 bristles or less	5	6	♀♀ No. of bristles 4 bristles or less	5	6
4.6*			4.0*	0.4*	—
4.6*			3.6*	1.0*	—
4.8*			3.1*	0.7	1.0*
3.6*	0.6*		2.3*	1.1	1.0*
3.2*	1.2		2.6*	1.1	0.7*
3.2*	0.8*		2.5*	0.5	—
3.2*	0.8	0.4*	2.4*	1.2	0.8*
3.2*	1.1	0.3*	2.0*	1.0	1.2*
2.8*	1.6		1.4*	1.1	1.9
2.4*	1.5	1.1*	1.8*	1.2	2.0
Average 1.24		—	—	0.99	1.95

* Values are minima; they represent the tails of the distributions and are not included in the averages.

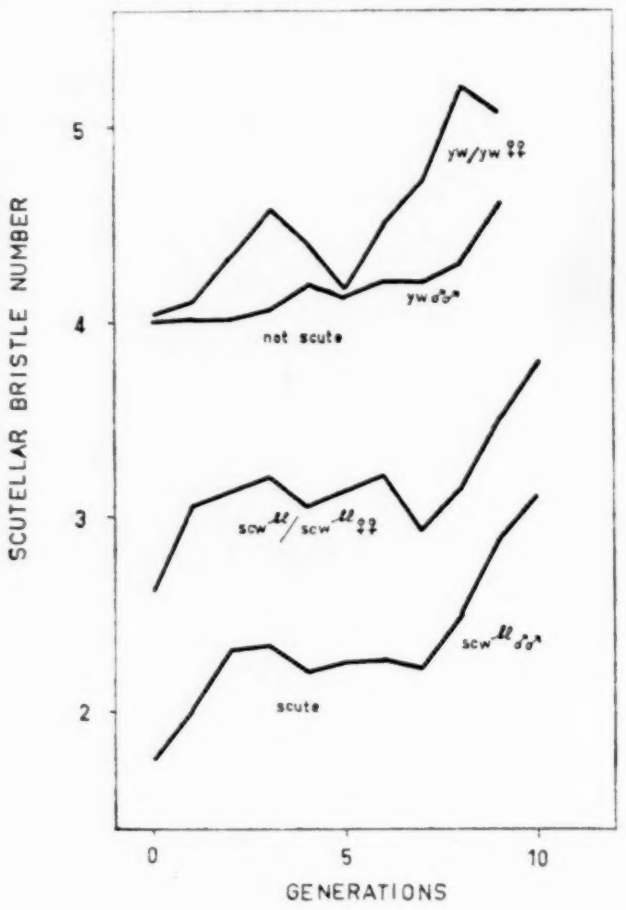


FIG. 5. Progress of selection for scutellar bristle number: above—in the *yw* selection line (homozygous not scute); below—in the *sc* w^{bl}/*sc* w^{bl} selection line (homozygous scute).

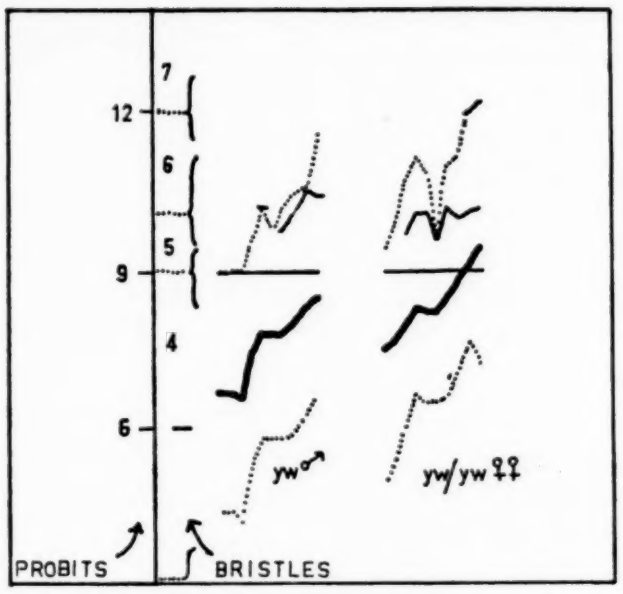


FIG. 6. Progress of *yw* selection line expressed in probits. Conventions as in figure 3.

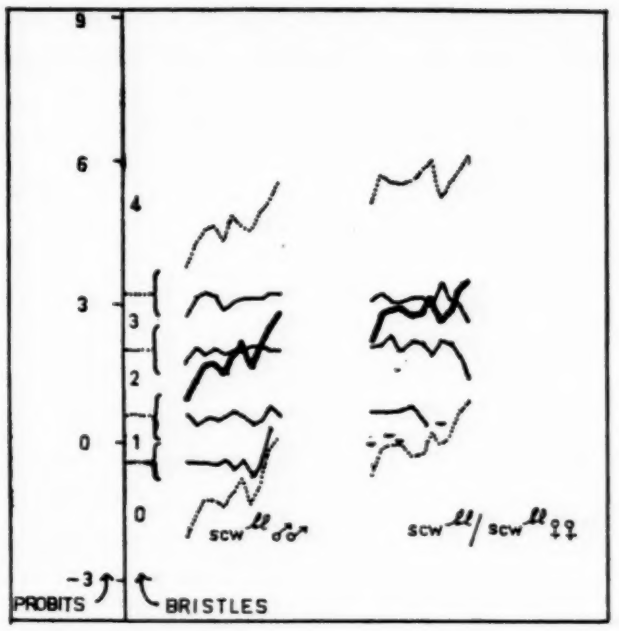


FIG. 7. Progress of *sc* w^{bl} selection line expressed in probits. Conventions as in figure 3.

not possible to follow genotypic movements through the 4-bristle class exactly; it has been assumed that the starting point as already argued is 3.4 units above the 3-bristle class line; a more or less straight line joins this point to the point where 5-bristle flies appear regularly. The point the mean genotypic value has reached by then is judged from the proportion of 5-bristle flies.

A more complete picture of the genotypic change required to change bristle numbers greater than four is given by the history of the *yw/yw* line which started from a female with five bristles; this is shown in figures 5 and 6 and tables 6 and 7. Once the mean bristle number started to move, females and males diverged, the bristle number increased rapidly, and soon reached seven in a few females. The genetic difference covered by the 5-bristle class corresponds to about one probit and, on very slim evidence (two flies), the 6-bristle class seems to cover about two probits.

The history of the homozygous scute line is shown in figues 5 and 7 and tables 8 and 9. The probit distance covered by different classes of bristle number in these lines is similar to that in the other groups; the main point of interest is that though towards the end of the experiment the

TABLE 8. *Progress of selection for the scutellar bristle number in the homozygous selection lines*

Generation	♂♂ No. of bristles					Mean	♀♀ No. of bristles					Mean
	0	1	2	3	4		0	1	2	3	4	
1	50	141	202	89	19	1.77	8	29	195	173	91	2.63
2	25	78	322	137	21	2.09	0	13	158	249	213	3.05
3	9	48	194	142	29	2.32	2	7	98	168	177	3.13
4	8	51	283	163	46	2.34	1	9	110	206	247	3.20
5	11	50	174	91	28	2.21	1	11	115	111	162	3.05
6	14	74	304	230	103	2.46	1	6	156	232	253	3.13
7	3	30	198	206	84	2.65	0	0	56	174	255	3.41
8	5	54	275	133	24	2.24	0	2	134	199	117	2.95
9	3	31	237	162	64	2.51	0	0	75	120	120	3.14
10	3	7	56	95	55	2.89	0	0	19	76	145	3.53
11	1	3	54	123	92	3.11	0	1	3	33	173	3.80

TABLE 9. *Probit distance occupied by bristle classes of flies of the homozygous selection lines*

Generation	♂♂ No. of bristles					♀♀ No. of bristles				
	0	1	2	3	4	0	1	2	3	4
1	1.6*	1.0	1.1	1.0	1.1*	0.7*	0.7	1.4	1.0	2.0
2	1.2*	0.8	1.5	1.2	1.1*	—	0.8*	1.4	1.1	2.5
3	0.8*	0.9	1.3	1.3	1.3*	0.2*	0.5	1.6	0.7	2.6
4	0.7*	1.0	1.5	1.1	1.5*	—	0.7	1.3	1.0	2.8
5	0.9*	1.0	1.3	1.0	1.4*	—	1.0	1.4	0.9	2.5
6	0.5*	1.2	1.3	1.0	1.8*	—	0.6	1.7	0.9	2.8
7	0.4*	1.0	1.4	1.1	1.9*	—	—	1.7*	1.1	3.0
8	0.6*	1.1	1.7	1.0	1.4*	—	0.4*	1.8	1.2	2.2
9	0.4*	1.0	1.6	1.0	1.8*	—	—	2.0*	1.0	2.4
10	0.4*	0.5	1.2	1.2	1.9*	—	—	1.2*	1.1	2.8
11	—	0.5	1.4	1.2	2.3*	—	—	0.5	1.2	3.5
Average	—	0.91	1.39	1.10		—	0.7	1.39	1.02	—

* Values are minima; they represent the tails of the distribution and so are incomplete classes and are not included in the average.

great majority of females has four bristles no fly has five, which confirms the view that a much greater genetic change is required to move through the four class than through any other. According to figure 4 all females and almost all males will have 4 bristles before any female has five.

So long as the genetic variation remains much the same in amount and distribution, the expression of the phenotype in probits is a genetic scale of similar value throughout; but it is arguable that the spread of a population after 22 generations of selection measures something very different from the spread at the start, quite apart from change of mean. I see no way at present of proving that this is or is not so. I am satisfied at this stage to call attention to the constancy of any given bristle class and the variation of bristle number in the high *yw* line. Any given bristle class is very constant, considering the size of population of each generation, except where the class is beginning to appear or disappear. On the borderline of appearance and disappearance, the presence or absence of one or a few bristles has an undue effect on the probit score of the borderline class. In the high *yw* line the 4-bristle class, which is the lowest, covers a much wider range of genotypes than any other, just as it does in the earlier experiments where it was the highest; further, as selection proceeds the 5-, 6- and 7-bristle classes come to occupy much the same pattern as the 1-, 2- and 3-bristle classes of the heterozygous selection lines. It would be too much to hope that there had been no change and that the left-hand scale of figure 3, which represents genotypic value, is identical from top to bottom. I believe, however, that it is nearly so and that, following Lush (1949) and others, one need not expect much change in genetic variance even after prolonged selection. Inbreeding is more likely to have interfered with genetic variance, but, in this experiment, the periodic crossing of the original ten separate lines may have done much to restore what was lost by inbreeding.

DISCUSSION

The way the phenotype reflects the genotype for scutellar bristle number is somewhat complex and it is not easy to find a suitable score in which to express what seems to underlie the phenotypic changes. I have assumed that when selection started the bristle scores of the two

scute genotypes reflected a set of genotypic values whose frequencies were approximately normally distributed, so that the interval between genotypic values at which one bristle number advanced to the next could be measured in probits. As the mean has increased from one bristle to five, it has been possible to scan genotypes reflected by bristle numbers from 0 to 7, and, on the assumption that the spread of the phenotype in the last generation covers the same genotypic distance that it did at the beginning, phenotypes have been given relative genetic scores. The resulting picture has been shown to be internally consistent and, insofar as it is a real picture, enables some conclusions to be drawn about the genetic control of scutellar bristles. The way the number of scutellar bristles reflects the genotype is very diverse; development seems to be canalized around four, and to some extent around two and possibly six bristles. It could be said that zero is also a canalized score, in that a wide belt of genotypes appear to have this score. If we were to draw a gene dose response curve it would have a vertical section at zero, a section approaching 45° between zero and two, a more vertical section through two, a second 45° section between two and three and then another vertical section at four, after which the steeper and flatter sections appear to continue to alternate.

This could be described in terms of gene interaction or epistasis, as has been done by Fraser (1959) for a hypothetical population living in an electronic computer, by plotting gene dosage deduced from the probit scores against the phenotype score and fitting a curve to the points. The coefficients of x, x^2 . . . etc. then show different levels of interaction. Such a description may well have advantages over the graphical one used here for some purposes. The points to be made, however, show up quite readily from the figures.

The interaction responsible for the changes in slope of the curve is clearly one between increments of genetic dosage and total dose; a gene increment which has shifted the *sc* male population in the heterozygous lines by two genotypic units has shifted the *sc/sc* female population two units also. But, whereas the male mean phenotypic score has risen nearly two bristles, the females have advanced only 1.4 bristles. The males are now slowing down as they approach four bristles and appear to be following the course taken by the females, but two genotypic units behind. The + genotypes at the beginning had no variation at all, so the action of the + gene with respect to the rest of the genotype was of an extreme epistatic type completely obliterating all the effects of the other genes. This position is being reversed and now the addition of the + gene reveals variation not visible in the *sc* genotypes, the *sc* gene thus beginning to act as though completely epistatic to the rest of the genotype. For example, I expect the *sc* gene in the *yw* selection line would reduce the mean to four bristles with little variation, from five with a full range. It is difficult to believe that this switch in epistatic effect of two alleles can result from direct interaction between particular genes; the whole picture is far more consistent with the supposition that at certain levels of genotypic value, genetic changes make much greater differences than at others, no matter what genes bring about the change in genotypic value. I will go further and say that in this particular character it appears to be the level of the phenotype, whether genetically or environmentally brought about, which fixes the effect of genetic changes. My reason is that, when for undetected causes the culture conditions are such that the whole Drosophila room drops in bristle number, the selection lines drop also and the pattern of numbers is consistent with the phenotypes attained in previous generations, although one must assume that the genotypes have changed. In this particular selection experiment, it seems that change in mean score has placed the popu-

lation on a part of the dose-response curve which has different characteristics from those at other mean scores, and has not altered the shape of this curve.

I do not want to suggest that it would be impossible to change the nature of the dose-response curve which must have been built up by differential survival in the past. In fact, preliminary results have shown that selection for and against variance in bristle number in lines with a mean number of about two has caused the lines to diverge, one having a variance nearly double the other. Dun and Fraser have shown that the effect of the tabby gene is to disrupt the canalizing genotype which is just what appears to be breaking down in my high variance selection line although it is really too early at this stage to interpret the reasons for increased and decreased variance. In the experiment reported here the alternative method of escaping canalization by developing the character on a different point of the scale has operated.

At first sight there is little reason for expecting a character like scutellar bristle number to be so strongly canalized: it is difficult to see how survival is going to depend on scutellar bristle number. It is quite reasonable to expect an orderly development of the thorax in general to be of survival value and, if development of bristles is somehow caught up in development of the thorax, it may share fortuitously regulation which has developed from advantages conferred by other anatomical structures. If so, one can expect destruction of canalization itself to result in loss of viability, but avoidance of it to leave viability unchanged. One can imagine avoiding canalization by bringing about processes essential to bristle formation outside the general control of thorax development. Suppose, for example, that there is a stage in development at which bristle formation comes under the general control of thorax development; then, by delaying the bristle-forming process, it may be postponed to a stage where it is outside the general control. Therefore it would be more variable, but with less time to operate, the process produces fewer bristles: this may be regarded as illustrating the action of scute. The selection back to normal timing restores four bristles and regularity. But when the + gene is reintroduced, normal timing is anticipated and so irregularity and number are both increased. If this illustration is the kind of way things are operating, it may also help to explain why interaction appears to be between genotype and phenotype rather than between gene and gene. The gene-dose is supposed to be shifting development into or out of a time period in which other developmental processes of overriding importance set the scene. Canalization is imposed by this latter system, and the shift of genotype merely moves bristle formation into or out of its sphere of influence. Breakdown of the canalization system itself would have far more serious effects.

If there are two ways in which canalization can be avoided, characters not associated with fitness may sometimes show non-additive genetic variance. Canalization is a product of natural selection, having advantage because it confers regularity and precision on development despite influences tending to deform the course of development. It will therefore always have a primary association with fitness. But other characters may be canalized merely through temporal or spatial association with the fitness character. A breakdown of canalization of the fitness character will always result in loss of fitness accompanying increase in variability, and as long as canalization persists, it will be accompanied by non-additive variation. But an associated character will only gain variability at the expense of fitness if the gain in variability results from breakdown in canalization of the fitness character: if it follows merely from a shift of a rather neutral character away from the influence of a fitness character, there is no reason why its fitness should alter. Such a character will have non-additive genetic variance so long as it is canalized and will

become additive as it moves out of the influence of canalization. The type of non-additive gene action shown here is due to interaction between genotype and phenotype and has been discussed by Fraser (1959).

Whatever the true explanation of the dose-response curve, it follows from the selection experiment I have described that the variance of a character is going to depend in all similar cases on the phenotypic level of the character, and the position of the population mean on the dose-response curve. Scutellar bristle number is very constant at zero and at four and relatively so at two. Insofar as heterozygotes will always be more likely to have characters nearer the mean than homozygotes, this is one reason for expecting the homozygotes of pure lines to be more variable than F_1's between pure lines.

Summary

Selection for scutellar bristle number in lines of *D. melanogaster* segregating for scute, by choosing scute males with high numbers as sires, raised the number of bristles in scute flies to four and the number of bristles in wild type sibs to six in extreme cases. A method of estimating the relative genetic change required to bring about different bristle changes is described. It is suggested that to move from three bristles to five takes eight times the genetic change that it does to move from one bristle to three. Bearings of the findings on canalization and non-additive genetic variation are discussed.

Literature Cited

Dun, R. B., and A. S. Fraser. 1958. Selection for an invariant character—'vibrissa number'—in the house mouse. Nature, **181**: 1018–1019.

Fraser, A. S. 1959. Simulation of genetic systems by automatic digital computers. 5. Linkage, dominance and epistasis. Proc. of the Int. Simp. on Biom. Genet., Ottawa (in press).

Lush, J. L. 1949. Animal Breeding Plans. 3rd ed., Iowa State College Press, Ames.

Waddington, C. H. 1953. Genetic assimilation of an acquired character. Evolution, **7**: 118–126.

——. 1957. The Strategy of the Genes. Allen and Unwin, London.

SOME ASPECTS OF EVOLUTION IN LAKE NYASA

GEOFFREY FRYER

East African Fisheries Research Organisation, P.O. Box 343, Jinja, Uganda

Received December 12th 1958

As a result of some two years' field work on the fauna of Lake Nyasa, and subsequent analysis of the information gained, a number of points relevant to the evolutionary phenomena shown by that fauna have emerged. Some of these have been mentioned in previous papers, particularly in one dealing with the ecology and evolution of some of the littoral fishes (Fryer, 1959), but several points, albeit disconnected, but none the less pertinent, remain to be presented. It is the purpose of the present paper to draw attention to these points.

(1) THE NON-DIVERSITY OF HABITATS

The fact that some of the great lakes of Africa are large and deep has led to the assumption that they provide a great diversity of habitats (de Beaufort, 1951). That this is not so in certain cases was, however, pointed out by Worthington (1954), who referred to the monotony of the shorelines of Lakes Rudolf and Kioga, and to the absence of dissolved oxygen at depths of 60–200 meters in certain other lakes. More recently, when referring to Lake Nyasa, Jackson (1955) suggested that "sufficient stress has not been laid . . . on the large variety of habitats present in a big lake such as Nyasa." With this I cannot agree, for, in spite of its great size, Lake Nyasa seems to provide a remarkably small assortment of habitats.

As in all lakes, save the very smallest and shallowest, Lake Nyasa is divisible into littoral, sublittoral and profundal regions and there is the usual pelagial zone —here of enormous extent and of typical uniformity. In spite of the great length of its shoreline the number of major littoral habitats in the lake is remarkably few; two types, rocky shores and sandy beaches, predominate. Some of the sandy shores are reed-fringed, others bare. This, however, is of scant importance to the majority of sandy shore species. Other major littoral habitats are few, and estuarine regions, occasional areas of local swamp, and even rarer shingly beaches cover all the other main types. Littoral macrophytic vegetation is very sparse; it is represented over much of the lake almost entirely by *Vallisneria,* and even this is absent from some exposed sandy beaches, and is completely absent from rocky shores, which support no higher plants whatsoever. Habitat differences related to the zonation of plants at different depths, which are so prominent a feature of many lakes, are scarcely recognizable in Lake Nyasa. The only vegetational change with increasing depth is usually the disappearance of *Vallisneria.*

Within each major littoral habitat there are of course microhabitats and/or niches (depending on the usage of these terms), but there is nothing to suggest that the number of microhabitats is any greater than in other lakes; in fact, the uniformity of conditions within each major habitat suggests that such microhabitats are relatively few (see also remarks on the restricted number of available feeding niches, p. 442).

The great maximum depth of Lake Nyasa (over 700 meters) suggests the existence of a series of habitats in the profundal region. However, at least 45% of the bottom area of the lake cannot be inhabited by aerobic organisms because it is completely and apparently permanently oxygenless, and, at least in parts, contains hydrogen sulphide. A considerable percentage of the remainder of the bottom is very nearly in this deoxygen-

ated condition. The maximum depth at which dissolved oxygen has been detected is about 250 meters and here the percentage saturation is always too low to permit most animals to survive. At a depth of 150 meters the amount of dissolved oxygen not infrequently falls below 1 part per million (Beauchamp, 1953).

It is not denied, however, that in water down to say 100 meters, a series of niches exists for exploitation. However, notwithstanding the presence of a few deep-water species of *Bathyclarias* (caught at depths as great as 70 meters) (Jackson, 1959), which in any case must eat to live and are therefore precluded from living at the greater depths which their tolerance of low oxygen tensions to some extent permits, it is true to say that if all the water below a depth of say 30 meters be discounted from consideration, the picture of the fauna of Lake Nyasa would scarcely differ from that which it presents in its entirety.

Even if a distinct "deep-water" fauna (at depths of say 70–120 meters) should be discovered in Lake Nyasa, as one has been in Lake Tanganyika (Poll, 1956a and b), the argument would in no way be altered insofar as the already known fauna and its habitats are concerned.

Further points in connection with the uniformity of conditions in Lake Nyasa, as compared with many other lakes, are the relatively slight change in temperature throughout the year, which perhaps militates against the development of seasonal faunas, and, more important, the small thermal gradient with increasing depth. In temperate lakes vertical zonation is reinforced, at least during the summer months, when most animals are active, by marked differences in temperature (a difference of 15° or 20° C. between water at depths of 1 meter and 30 meters is by no means unusual). Such striking thermal gradients exist also in warm monomictic (subtropical) lakes (Yoshimura, cited by Hutchinson, 1957). In Lake Nyasa, as revealed by Beauchamp's data, such temperature gradients are almost inconsequential, for even at times when thermal stratification is most marked the difference in temperature between the upper layers of water and that at 200 meters is only about 6° C., and frequently the habitable zone is more or less homothermal.

It is therefore apparent that Lake Nyasa does not offer the diversity of habitats that some have supposed.

(2) Qualitative and Quantitative Poverty of Certain Animal and Plant Groups

Of the Nyasan fauna only the fishes and one or two other groups have been studied in detail by systematists. However, ecological studies in the littoral zone have shown that the number of species of invertebrates in certain groups is surprisingly low, thus to a large extent bearing out Moore's statement made as long ago as 1903 that the fauna of Lake Nyasa consists of "fishes and molluscs." Even molluscs are by no means everywhere plentiful, and on rocky shores in the northern part of the lake they appear to be completely absent. Of the Crustacea the order Cladocera is almost entirely absent from the littoral zone, at least in the northern part of the lake. The number of species of the larger insects is also surprisingly low, and the species that do occur are by no means so abundantly represented as in many lakes. The paucity of the higher plants has been referred to above. On the other hand, a few groups, such as lithophilic chironomid larvae and ostracods, may occur in enormous numbers in certain microhabitats (Fryer, 1959).

Thus it is apparent that the fishes, which exhibit the most remarkable speciation of all the groups in Lake Nyasa, have not had a particularly diverse assortment of foods at their disposal.

It is relevant to record here that the "lake flies" which often emerge in vast swarms from Lake Nyasa are not, as is generally assumed, chironomids, but chaoborids, apparently of one species only,

Corethra edulis Edwards. They were referred to as chironomids by Beauchamp (1940) whose statement was utilized as the basis of a supposition by Brooks (1950) in a well-known review. As the larval habits of these two groups differ fundamentally, the one being benthic and the other planktonic, this erroneous idea conveys a completely wrong impression of the hydrological and biological regimes of the deeper layers of Lake Nyasa, and calls for correction.

(3) Proof of Statements (1) and (2) and Their Evolutionary Significance

Since many species of fishes are present in Lake Nyasa (particularly in the family Cichlidae, of which more than 180 species are now known), the occurrence of many species per habitat should follow if, as has been suggested in (1), there are few major habitats in the lake. This is indeed true. Further, if, as suggested in (2), the number of available foods is few, then the utilization of the same kind of food by several species is to be expected. Again this is true. (Details of the co-existence of many species in the major littoral habitats, and of their feeding habits, are given in Fryer, 1959.)

The significance of these facts to the evolutionary biologist is that one cannot attribute the remarkable speciation of the cichlid fishes of Lake Nyasa to the availability of a diversity of habitats for colonization, nor to the availability of a diverse series of foods that would allow each species to fill a different feeding niche.

Perhaps, as a corollary to the second conclusion, attention may be drawn to the remarkable habit of feeding on the scales of other fishes that has arisen at least three times among the Nyasan cichlids—in *Corematodus,* in *Genyochromis,* and in a new species possibly referable to *Haplochromis* or perhaps meriting the erection of a new genus. This habit may have arisen partly as a result of the availability of only a few different types of food and partly because so many other fishes were available for exploitation.

(4) The True Importance of Size

Although the great size and depth of Lake Nyasa have not resulted in the provision of a great diversity of habitats as some have supposed, these factors have not been without importance in evolution. First, as was pointed out by Worthington (1937) and is now generally accepted, the great depth of Lake Nyasa enabled the basin to retain water, and with it an aquatic fauna, during the arid interpluvial periods of the Pleistocene that resulted in the dessication of shallower lakes elsewhere in the continent. In addition size *per se* has also been of importance.

As is pointed out elsewhere (Fryer, 1959), the relatively few major habitats

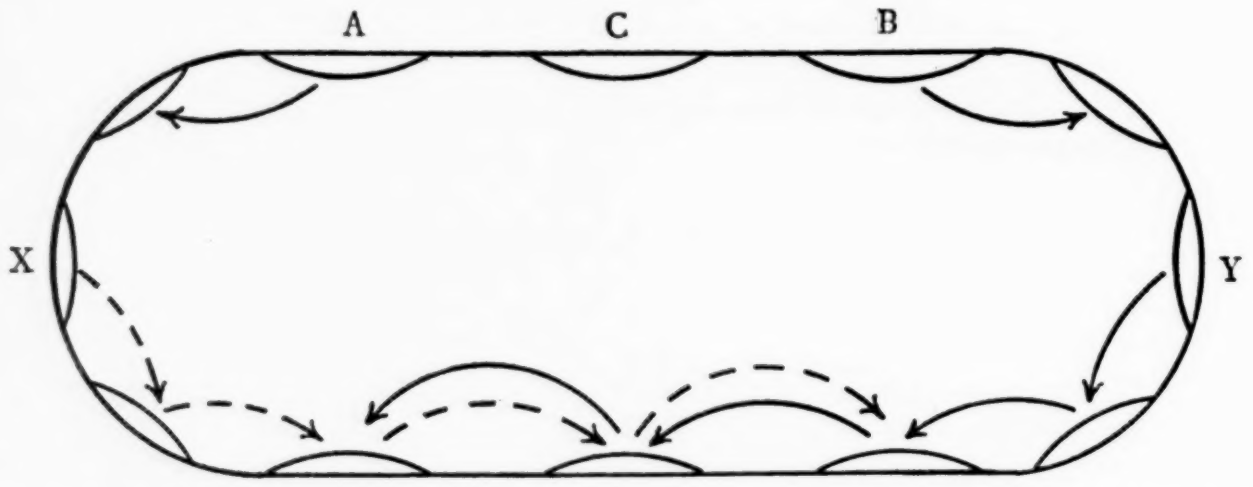


Fig. 1. Diagrammatic representation of Lake Nyasa showing how one type of habitat (e.g. rocky shore) is reproduced many times to give a series of similar but isolated habitats. Arrows indicate movements of fishes from original centers. For details see text.

are fragmented: rocky shores and sandy beaches alternate. Now consider a given stock of an organism that in the past became distributed around the whole or part of the lake in suitable habitats as a result of the various changes, some of them possibly cataclysmic, that have taken place during the history of the basin. Representatives of this stock may have begun to specialize in different directions in areas remote from (or near to) one another, e.g. at X and Y (or A and B) in figure 1. Both specializations, although perhaps different, may have been successful. Now the time taken to overcome the various barriers between the two and to cover the distance involved would, it is postulated, sometimes be sufficient to allow the two populations to become sufficiently distinct to prevent interbreeding when they (e.g. populations X and Y in figure 1) eventually met. Such a state of affairs is comparable with the Lesser Black-Backed—Herring Gull circumpolar cline in the northern hemisphere. Even in cases such as A and B where "swamping" could take place at C, the outwardly migrating mutants could become more and more distinct and separated by ever increasing distances until they eventually overlapped. Evolution of this type could not take place so easily in a smaller lake where the time required to cover the smaller distances and surmount the less numerous barriers would be less.

The size of the lake is such that even planktonic organisms can be isolated in different regions, although the pelagial waters in which they live extend throughout the lake with no apparent barriers. The fact that this is probably a reflection of different types of lake morphometry acting via the trophic regime in different areas in no way alters the fact that size is ultimately the governing factor. Thus the endemic copepod *Diaptomus* (*Thermodiaptomus*) *mixtus* Sars and the non-endemic cladoceran *Daphnia lumholtzi* Sars have been found in abundance at the southern end of the lake but not in the north (Fryer, 1957). The fact that such organisms, which can easily be dispersed by currents, can be isolated in this way suggests that those animals that are capable of resisting such chance dispersal are even more likely to be isolated as a result of the great size of the lake.

(5) The Role of Predation—Further Suggestions

The suggestion that lakes whose cichlid fish faunas are subject to considerable predation show less speciation than those in which predation is slight was put forward by Worthington (1937), who still holds this view (1954). His suggestion that predation is slight in Lake Nyasa was questioned by Fryer and Iles (1955), who showed that predacious fishes are particularly numerous in this lake. Later it was suggested that the predators, by retarding the growth of populations of non-predatory species, help to prevent competition between them for the available food, and perhaps in some cases for living space, and consequently assist in the survival of large numbers of species in a given habitat (Fryer, 1959). It remains to be seen which of these diametrically opposed views will prove to be correct. Here some further aspects of predation that appear to support the second view are put forward:

(a) It has been emphasized how, in the littoral zone, a given species of fish usually is rigorously restricted to a given habitat. Now, if for any reason, a fish from say a rocky shore should attempt to cross a strip of sand or vice versa it would, as a result of its coloration and ill-adapted escape reaction in that habitat, be particularly easy prey for a predatory fish. Worthington (1954) said exactly the same thing, viz., "Where predators are continually on the prowl, the lesser forms, adapted to their particular ecological niches can depart from them to colonise new micro-environments or to breed with their neighbours only on pain of death," but used this as evidence that predation has had a marked retarding influence on speciation. This is surely a

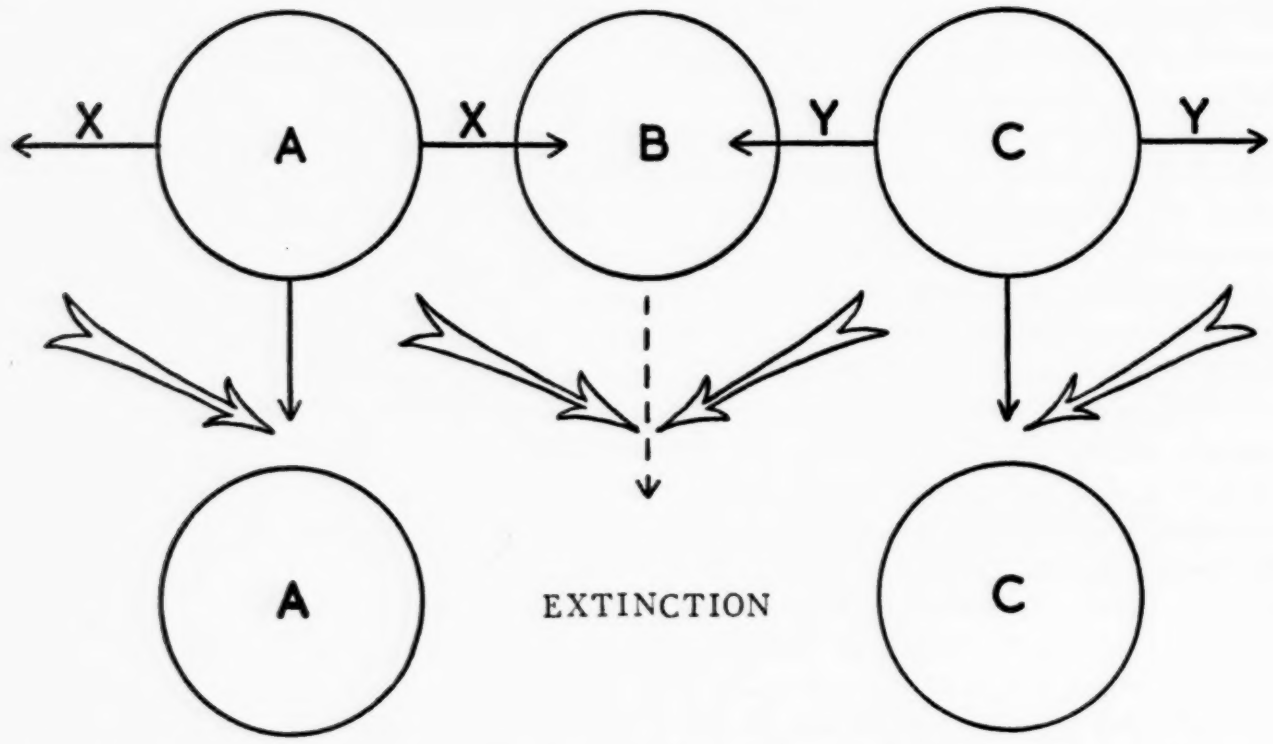


FIG. 2. Diagrammatic representation of how predation can facilitate speciation by the elimination of hybrid populations. A and C are the original populations specializing in directions X and Y respectively. The vertical arrows represent the passage of time and the broad arrows predation. For further explanation see text.

wrong interpretation. By restricting species to particular habitats the predators in fact assist in keeping them isolated in numerous discontinuous populations and therefore undoubtedly favor rather than restrict speciation. If this be so, then one can readily understand why the presence of the large number of predacious species shown by Fryer and Iles (1955) to occur in Lake Nyasa is not incompatible with the existence there of the greatest total of cichlid fishes of any African lake.

(b) It must be generally agreed, and is agreed to by Worthington (1954), that ill-adapted mutants of the fishes of the various habitats will more readily fall victims to predators than their better-adapted brethren. Such a belief is indeed implicit in the concept of natural selection. In the absence of predators some of these ill-adapted mutants would survive (though others would be eliminated by other means), and this would lead to a reduction in efficiency of the species concerned, and thus enhance the likelihood of elimination by competition. Predatory activity of this kind therefore surely assists in the survival of species rather than in their elimination.

(c) Another way in which predation can assist in the process of speciation can be understood by reference to figure 2. Consider a stock of fishes split into two (it could be more) populations as at A and C. Now suppose that population A begins to specialize in a certain direction X and population C begins to specialize in a different direction Y. Members of each of these populations would eventually move outwards from their centers of origin as shown in the figure. Now, if genetical or behavioral differentiation had not proceeded to such a stage as would prevent interbreeding of these stocks, a hybrid population in which the antagonistic X and Y tendencies resulted in the production of ill-adapted offspring could arise at B. Such an ill-adapted hybrid population would be more easily eliminated by predation and competition than either of the parent stocks, and such elimination would prevent the swamping and mixing of the X and Y tendencies which could then continue to increase

until ultimately they became characteristic of distinct species between which gene flow was impossible. Here again, therefore, the role of predation, which acts as a selective sieve, would be to favor rather than to restrict speciation.

All these suggestions, like those put forward elsewhere, point to the conclusion that the presence of predacious fishes in Lake Nyasa, and in other lakes, has probably hastened and assisted the speciation of the non-predatory species.

(6) The Restricted Radiation Patterns of the Cichlid Fishes

The overall picture of the adaptive radiation shown by the cichlid fishes of Lake Nyasa is one of completeness. Every available niche appears to have been filled. The means whereby this filling of niches has been accomplished, however, seems, at least in part, to be somewhat different from that generally accepted as explaining the ecological evolution of related species. Thus, analysis of the ecological data reveals the existence of a number of morphological types, some of generic and one of suprageneric standing, each of which has its own ecological preferences. Radiation of each of these particular types tends to be restricted within certain rather narrow limits. The outstanding example of this is that of an assemblage of 9 genera comprising some 27 species that form a compact morphological group whose members are more closely related to each other than to the species of any other genus in the lake. Although the members of this group show some very striking trophic adaptations, many of which are described elsewhere (Fryer, 1959), adaptive radiation has in fact been rather restricted in that all these species are to a greater or lesser extent confined to rocky shores, most of them rigorously so, and very many of them take exactly the same kind of food, namely, algae scraped from the rocks.

Among these rock-frequenting species identical food preferences are often exhibited both by the various members of a single genus and by the members of several genera. For example, within this group is one genus, *Pseudotropheus,* comprising 12 known species, of which at least 8 feed exclusively on lithophilic algae, as do members of the genera *Labeotropheus* and *Petrotilapia.*

The same phenomenon is exhibited at a generic level by other fishes of the lake. For example the genus *Rhamphochromis,* with at least 8 species, consists exclusively of active piscivorous species that frequent open water just offshore. Similarly the 24 or more species of *Lethrinops,* though showing a certain amount of trophic diversity, seem to be confined to sandy shores, where they feed on benthic invertebrates, usually insect larvae. More ecological information on this genus is needed, but it can at least be stated that not a single species was found on a well-studied rocky shore in the northern part of the lake during a comprehensive survey of its fauna, though several species occurred on an adjoining sandy beach.

The genus *Haplochromis,* which has given rise to rather more than 100 species in Lake Nyasa does not, at first sight, appear to conform to the pattern described above, for its members have colonized a variety of habitats and have evolved widely differing specializations. However, within this genus, whose limits are at present somewhat vague and certainly wider than those of most if not all other Nyasan genera, a certain amount of group-specialization of the type described above has taken place. This is shown most strikingly by an assemblage of species known collectively as the "utaka," that have taken to feeding on zooplankton and that are so similar morphologically as to render identification of some species distinctly difficult. Mr. T. D. Iles, who is making an intensive study of this group of fishes, has so far been able to recognize at least 16 species with certainty. He tells me that when his studies are com-

plete this total will almost certainly be increased. I am most grateful to Mr. Iles for this as yet unpublished information.

This type of radiation is quite different from that found in many other animal groups where, as in the passerine birds surveyed by Lack (1944), closely related species usually occupy separate habitats or regions or, if they co-exist, tend to take different foods. The latter type of radiation is indeed found even among the cichlid fishes of African lakes, for Greenwood (1958) reported the existence among the 70 species comprising the species flock of *Haplochromis* in Lake Victoria, of several morphologically similar species-pairs one member of which lives inshore on hard bottoms, the other offshore on soft bottoms, which implies that radiation took place by the colonization of new habitats during specific divergence. Such an evolutionary history during which the fishes radiated into a variety of habitats and began to take different foods seems to have been tacitly assumed by theorists seeking to explain the evolution of all the cichlid fishes of African lakes before sufficient ecological data were available. This, however, certainly does not apply to some of the rock-frequenting, algal-eating species of *Pseudotropheus* and allied genera, nor to some of the feeders on chironomid larvae among the sand-loving species of *Lethrinops,* nor to the numerous open-water plankton feeding "utaka," of Lake Nyasa. It seems probable also that a similar speciation pattern to that shown by these cichlids of Lake Nyasa will apply in large measure in Lake Tanganyika, which is morphometrically similar to Lake Nyasa and has a basically similar cichlid fauna.

The type of radiation described above, while not unknown elsewhere in the animal kingdom, seems to be distinctly unusual and I know of no case so striking as the ones described. On the contrary cases are recorded where infraspecific taxa frequent different habitats. For example, there are prairie and forest *races*

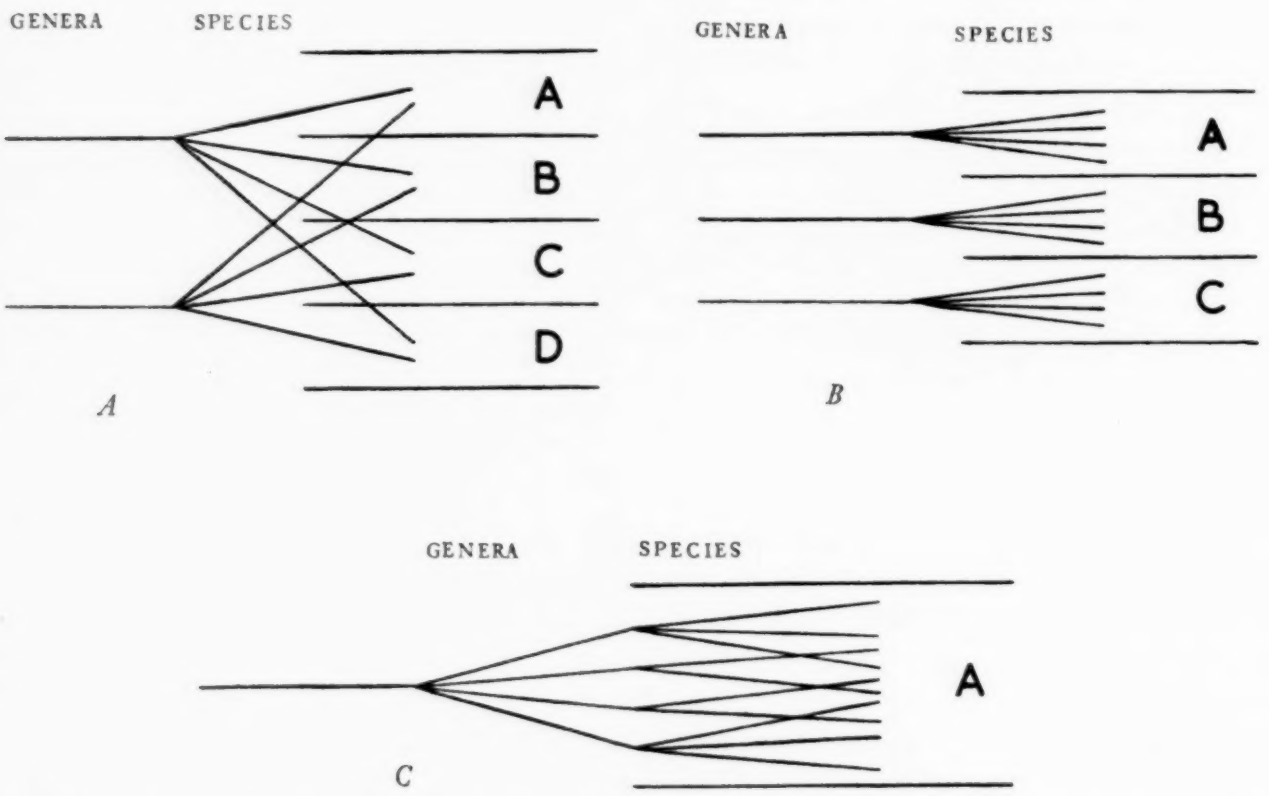


FIG. 3. Patterns of adaptive radiation. In each diagram the bands designated by the capital letters represent habitats: (A) The type of adaptive radiation typical of many groups of animals. (B) Adaptive radiation as exhibited by certain cichlid fishes of Lake Nyasa. (C) Adaptive radiation as exhibited by a group of rock-frequenting cichlid fishes (the "mbuna") of Lake Nyasa.

of the deermouse *Peromyscus maniculatus*.

(7) The Significance of Lake Level Fluctuations

The level of Lake Nyasa is not stable but is subject to annual and longer-term fluctuations. Possible influences of these fluctuations on the evolution of fishes were suggested by Trewavas (1947) and by Lowe (1952), and further points are mentioned by Fryer (1959). A rather important point which has not yet been sufficiently stressed is the influence of these fluctuations on the basic food supply of the littoral zone which is, faunistically, both qualitatively and quantitatively the richest zone of the lake. On sandy shores *Vallisneria* and its epiphytic algal flora are the most important basic foods, at least in the northern part of the lake. Now sandy shores often shelve quite gently near the shoreline then pitch steeply downward. As was observed on one such sandy beach (Fryer, 1959), a fall in the lake level may result in a serious depletion of this food on the gently sloping part without any possibility of an equally productive flora developing on the steeper slope, because of the limitations imposed by depth on the photosynthetic activities of the plants. Such a state of affairs could lead to an intensification of competition among the fishes utilizing these primary food sources either directly or indirectly; to the elimination of the less successful individuals or species; or to migration, for which, under normal relatively stable conditions there is very little tendency among most species.

(8) Plankton Feeding and Shoaling in Nyasan Fishes

The adoption of plankton feeding by Nyasan fishes seems to have gone hand in hand with the habit of shoaling. This is readily seen by a consideration of the plankton-feeding fishes of the lake. The small cyprinid *Engraulicypris sardella* (Günther), which subsists entirely on plankton (including occasional gluts of pupae of the midge *Corethra edulis* Edwards), is a good example, for where one specimen is found one usually finds thousands.

Numerous cichlids exhibit the same phenomenon. The most striking case is provided by the complex group of *Haplochromis* species known in the vernacular as the "utaka." All these appear to be plankton-feeding and shoaling species, yet none of the numerous other species of *Haplochromis* of the lake, which occupy a diversity of feeding niches, shoal.

A particularly instructive series showing how the degree of assumption of one habit is associated with a corresponding development of the other is that of the *Tilapia* species of the lake. Neither of the inshore species, *T. melanopleura* Duméril and the endemic *T. shirana* Boulenger, is a plankton feeder and neither shoals, but the endemic *T. saka* Lowe and *T. squamipinnis* (Günther), which inhabit more open water and feed partly on plankton, show a marked tendency to shoal, while the truly open-water plankton-feeding *T. lidole* Trewavas shoals throughout life (Lowe, 1952, and personal observations).

A particularly pertinent case is that of *Cynotilapia afra* (Günther), whose phyletic relationships are clearly with a group of littoral rock-frequenting fishes, none of which shoal. This species has taken to feeding on plankton but is still not emancipated from rocky shores (Fryer, 1959), yet, like the other plankton-feeding fishes of the lake, it shows a marked tendency to shoal.

To some extent this habit might be regarded as an example of ecological neoteny. This can be explained by reference to *Haplochromis kiwinge* Ahl. In its very early stages this species is brooded in the mouth of the female parent, and a dense shoal of young at this stage of development has been watched in nature feeding on particulate matter, presumably plankton, while guarded by the female (Fryer, 1956). Later in life *H. kiwinge* frequents rocky shores and,

while more catholic in its tastes, still feeds largely on zooplankton (Fryer, 1959). At this stage it tends to move around in groups, though as its size increases still more and it becomes more omnivorous it tends to become solitary.

The phenomenon of a change in diet from plankton to some other food being accompanied by a breakdown in shoaling habits is not uncommon among freshwater fishes generally. In some cases the cessation of shoaling is probably imposed by necessity when the diet is changed, for not all habitats could withstand the effects of large shoals of fishes demanding the same kind of food. The pelagial zone and the plankton which it produces impose no such limits and the habits of immaturity can be retained, as can the feeding habits.

A factor that is perhaps not unconnected with the maintenance of the shoaling habit but which, as is shown by the incipient shoaling behavior of *Cynotilapia afra*, has probably contributed but little to its evolution, is the tendency for shoaling species to congregate at certain points. In Lake Nyasa echo sounding has revealed the existence of shoals of plankton-feeding *Haplochromis* in areas where submerged rocks approach the surface and where the African fishermen say there are currents. Similar echo traces involving plankton-feeding fishes are obtainable in comparable situations in other African lakes (e.g. see Capart, 1955). This seeking out of particular areas where plankton is presumably obtainable with a minimum of effort will tend to concentrate pelagic fishes and thus reinforce the shoaling impulse.

(9) The Importance of Mouth-Brooding Among the Cichlid Fishes

The presence of very dense populations of fishes in the littoral zone of Lake Nyasa and particularly on rocky shores (Fryer, 1959), considered in conjunction with aquarium observations which have shown that even the herbivorous members of these communities eagerly devour eggs and young of their own and other species when opportunity offers, suggests that considerable advantages are conferred on those species which brood their eggs and young in the mouth; that is on the cichlids. Indeed it is difficult to conceive of unprotected eggs shed on a rocky shore surviving for more than a few minutes unless secreted in inaccessible crannies. Fishes that produce such eggs are therefore virtually precluded from breeding in this habitat, and in fact of the six non-cichlid species that frequent rocky shores in the northern part of the lake during the whole or part of their life history four are either definitely known to breed elsewhere or are suspected of doing so. Of the other two *Mastacembelus shirana* Günther is a small skulking fish of eel-like form that inhabits cracks and crevices inaccessible to other species of the habitat, and may well deposit its eggs in such situations. The breeding habits of the other, *Labeo cylindricus* Peters, are not yet known, but other species of this genus both in Lakes Nyasa and Victoria ascend rivers and streams to spawn (Lowe, 1952; Fryer and Whitehead, 1959).

The same state of affairs holds good for certain other habitats such as sandy shores for, as shown in (3), the number of species per habitat is high, though perhaps nowhere are the difficulties attendant on "free spawning" so great as on rocky shores. As on rocky shores some of the non-cichlids of sandy shores, e.g. *Labeo mesops* Günther, are known to run up rivers to spawn.

Relevant to this matter is the fact that at present many of the non-endemic species of the Nyasa basin, which are almost always non-cichlids, are, as Jackson (1955) has pointed out, confined to rivers, lagoons, and other situations fringing the lake, and can scarcely be considered as truly lacustrine. Even though the breeding habits of these species are often unknown one can at least be sure that they are not mouth-brooding fishes.

It is also interesting to note that the

two cichlid species of the Nyasan area which are not mouth brooders, namely *Tilapia melanopleura* Duméril and *T. sparrmani* A. Smith, which, by virtue of their wide distribution in Africa, must be regarded as very successful species, have met with little success in colonizing Lake Nyasa. *T. melanopleura* occurs only in certain weedy lagoons connected with the lake and in small numbers in a few sheltered regions of the littoral zone, and *T. sparrmani* occurs only in lagoons and rivers and has not been recorded from the lake proper. This of course may be due in large part to the lack of suitable habitats for these two species in the lake, but more significant is the fact that it is not from such egg-guarding species but from those forms which developed mouth-brooding that the cichlids which occupy the various habitats of Lake Nyasa today have been derived.

Such facts as these point to mouth-brooding as having played an important part in the biological success of the cichlids of Lake Nyasa. They also go a long way towards explaining why some of the habitats of the lake can support dense sedentary populations of fishes and why these populations are composed largely of mouth-brooding cichlid fishes.

(10) Brood Size, Egg Size, and Habitat Among the Cichlidae

Data concerning the size of the brood produced by the Nyasan cichlids are still too scanty to permit the making of many deductions, but in view of the interest recently aroused by Lack in the ecological and evolutionary significance of allied phenomena, and particularly in the clutch size of birds and the litter size of mammals (see Lack, 1954, for review), brief mention of one point is merited.

The habitat in which most predation on the very early stages of development is to be expected is that in which the population density is greatest; that is, on rocky shores. Under such conditions selection may be expected to favor those species producing a few large eggs which can be brooded until the young have attained a relatively large size. It is quite conceivable that survival will be greater in small broods that receive prolonged protection than in larger broods that are liberated at an earlier and more vulnerable stage of development—a state of affairs analogous to the often greater survival of young from small than from large clutches of eggs in birds because of the inability of the parent to feed more than a limited number of chicks. It is interesting therefore that available data suggest that the group of genera of fishes most characteristic of rocky shores (the "mbuna") produce the smallest number of eggs per clutch of all the Nyasan cichlids and, furthermore, these eggs are both relatively, and apparently absolutely, larger than those produced by the often larger cichlids of other habitats (Fryer, 1959).

Data on the brood size of the cichlids of other habitats, while scanty, indicate that, in general, broods are larger than in the rocky-shore species. For example, the figures given by Lowe (1955) for the number of ripe ovarian eggs produced by three closely related endemic species of *Tilapia* indicate that in these species, which live under much less crowded conditions than do the rocky-shore species, the number of eggs brooded is, at a conservative estimate, upwards of six times the number brooded by the rocky-shore species.

General observations on cichlids frequenting sandy shores, where populations are less dense than on rocky shores, but where the young are more exposed to predation than are the young of some of the species of *Tilapia* mentioned above, which brood their young among weeds, suggest that these species produce more eggs than the former but less than the latter, but more details are required.

The above data, while highly suggestive of an adaptive correlation between egg size, brood size and habitat, require further elaboration, particularly where complicating factors have to be considered. For example, in *Tilapia lidole* Trewavas

a few young are retained to a large size, presumably with the shedding of many smaller individuals—a fact perhaps indicative of a recent change to more open-water brooding conditions than those experienced by its relatives, and of incomplete adaptive changes to these conditions.

(11) The Importance of Specialization in Intralacustrine Speciation

Freshwater fishes are, in general, relatively unspecialized. This can be correlated with the transient nature of most freshwater environments (Larkin, 1956). Two aspects of this lack of specialization are an ability to exist under a wide range of environmental conditions (e.g. see any general work on British freshwater fishes) and the capacity of many species to utilize a wide range of foods (e.g. see Hartley, 1948). By way of contrast, many of the fishes of Lake Nyasa are extremely specialized for life in one particular environment and especially for the collection of one particular kind of food. This specialization seems to be intimately bound up with speciation. The relative permanence of certain environments in the lake is an incentive to specialization: the more specialized a species becomes, the more successful it is likely to be in a particular environment. Perhaps even more significant from an evolutionary point of view is the fact that the more specialized a species becomes, the more it becomes restricted to a particular environment and the less likely it is to venture into habitats to which it is ill-adapted. In other words specialization has led to the state of affairs described in detail elsewhere where most species exist in numerous discrete, isolated populations of various sizes—ideal conditions for the operation of allopatric speciation.

Summary

A number of facts and speculations relating to evolutionary phenomena within the basin of Lake Nyasa are put forward.

It is shown that, in spite of its great size, Lake Nyasa does not provide either a great diversity of habitats or of different foods for the fishes that exhibit the most striking speciation of all the groups in the lake. Thus neither the existence of many habitats nor of a wide choice of foods can be called upon as means whereby this speciation could have been accomplished.

The great size of the lake, enabling isolation by distance to become effective, is believed to have played an important part in permitting the evolution of numerous species.

Various aspects of predation, supplementing evidence previously put forward, suggest that, contrary to the view of Worthington, the presence of numerous predators has facilitated and not impeded speciation.

Although the cichlid fishes fill numerous niches, adaptive radiation has been restricted in that groups of related species, or even genera, frequently co-exist in the same habitat and often utilize similar foods. Examples of this type of radiation are given and comparison is made with the adaptive radiation more usually found elsewhere in the animal kingdom.

Some new considerations, in addition to those already put forward by previous students of the lake, indicate that lake-level fluctuations have had a bearing on the speciation of the fishes.

The evolution of plankton-feeding among the fishes of Lake Nyasa seems to have been invariably accompanied by the adoption of the shoaling habit. Examples of this, including an incipient case, are given. The suggestion is put forward that the shoaling habit is an example of "ecological neoteny."

The fishes showing the most remarkable speciation in the lake are those of the family Cichlidae which, with only two known exceptions, neither endemic, are mouth-brooders. The habit of mouth-brooding is believed to have been important in the evolution of the many endemic species, and in permitting their co-existence in dense multispecific populations.

Preliminary data suggest a distinct correlation between the size of the eggs produced, the number of young brooded by the female, and the habitat frequented by any given species of cichlid fish. Those species living in the most densely populated habitats produce few large eggs and rear small broods of young to a relatively large size, while species in less densely populated habitats produce larger numbers of relatively smaller young from smaller eggs.

Relatively stable conditions prevailing over a long period of time have apparently provided an incentive to specialization to the fishes of Lake Nyasa such as is absent in most lakes which, geologically speaking, have but a short life span. This specialization had the inevitable consequence of restricting individual species to very definite habitats, thus rendering isolation of populations by even small barriers of unsuitable terrain an easy matter. Such conditions have been conducive to allopatric speciation.

Literature Cited

Beauchamp, R. S. A. 1940. Chemistry and hydrography of Lakes Tanganyika and Nyasa. *Nature Lond.*, **146**: 253–256.

——. 1953. Hydrological data from Lake Nyasa. *J. Ecol.*, **41**: 226–239.

de Beaufort, L. F. 1951. Zoogeography of the Land and Inland Waters. viii + 208. Sidgwick & Jackson. London.

Brooks, J. L. 1950. Speciation in ancient lakes. *Quart. Rev. Biol.*, **25**: 30–60; 131–176.

Capart, A. 1955. L'échosondage dans les lacs du Congo Belge. Techniques et résultats acquis. *Bull. Agric. Congo Belge*, **46**: 1075–1104.

Fryer, G. 1956. Biological notes on some cichlid fishes of Lake Nyasa. *Rev. Zool. Bot. Afr.*, **54**: 1–7.

——. 1957. Freeliving freshwater Crustacea from Lake Nyasa and adjoining waters. Part III. General remarks with notes on certain Malacostraca and Ostracoda. *Arch. Hydrobiol.*, **53**: 527–536.

——. 1959. The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with especial reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. *Proc. Zool. Soc. Lond.*, **132**: 153–281.

Fryer, G., and T. D. Iles. 1955. Predation pressure and evolution in Lake Nyasa. *Nature Lond.*, **176**: 470.

Fryer, G. and P. J. P. Whitehead. 1959. The breeding habits, embryology and larval development of *Labeo victorianus* Boulenger (Pisces, Cyprinidae). *Rev. Zool. Bot. Afr.* **59**: 33–49.

Greenwood, P. H. 1958. Evolution and speciation in the *Haplochromis* (Pisces, Cichlidae) of Lake Victoria. *15th Int. Cong. Zool.*, Sect. 2. Paper 2: 1–3.

Hartley, P. H. T. 1948. Food and feeding relationships in a community of freshwater fishes. *J. Anim. Ecol.*, **17**: 1–14.

Hutchinson, G. E. 1957. A treatise on Limnology. Vol. I. xii + 1015. Wiley, New York.

Jackson, P. B. N. 1955. A new fish of the genus *Clarias* Gronov. from Lake Nyasa, with notes on the distribution of the Clariidae and other catfishes in the lake. *Proc. Zool. Soc. Lond.*, **125**: 681–684.

——. 1959. Revision of the clariid catfishes of Nyasaland, with a description of a new genus and seven new species. *Proc. Zool. Soc. Lond.*, **132**: 109–128.

Lack, D. 1944. Ecological aspects of species formation in passerine birds. *Ibis*, **86**: 260–286.

——. 1954. The Natural Regulation of Animal Numbers. viii + 343. Oxford Univ. Press.

Larkin, P. A. 1956. Interspecific competition and population control in freshwater fish. *J. Fish. Res. Bd. Canada*, **13**: 327–342.

Lowe, R. H. 1952. Report on the *Tilapia* and other fish and fisheries of Lake Nyasa. 1945–47. *Col. Off. Fish. Pub.* 1. No. 2. 126 pp.

——. 1955. The fecundity of *Tilapia* species. *E. Afr. Agric. J.*, **21**: 45–52.

Moore, J. E. S. 1903. The Tanganyika Problem. xxiii + 371. Hurst & Blacket. London.

Poll, M. 1956a. Ecologie des poissons du Lac. Tanganika. *Proc. 14th Intern. Cong. Zool.*, 465–468.

——. 1956b. Poissons Cichlidae. *Explor. Hydrob. L. Tanganika*, **3**: Fasc. 5B, 619 pp.

Trewavas, E. 1947. Speciation in cichlid fish of East African lakes. *Nature Lond.*, **160**: 96–97.

Worthington, E. B. 1937. On the evolution of fish in the great lakes of Africa. *Int. Rev. Hydrobiol.*, **35**: 304–317.

——. 1954. Speciation of fishes in African lakes. *Nature Lond.*, **174**: 1064–1067.

CALL DIFFERENTIATION IN A COMPLEX OF SEVEN SPECIES OF *CRINIA* (ANURA, LEPTODACTYLIDAE)[1]

MURRAY J. LITTLEJOHN [2]

Zoology Department, University of Western Australia, Nedlands, Western Australia

Received December 31, 1958

The problem of the origin and operation of reproductive isolating mechanisms is one for which additional information is desirable. Of basic importance to a study of this subject are data on actual or potential isolating mechanisms, with emphasis on geographical variation, divergence in allopatry, and finally operation in sympatry. If possible the information should be derived from a group of closely related species and the data should be handled objectively and quantitatively.

Much study has been done on the isolating mechanisms of anurans. In general it appears that the mating call, usually uttered by the male, is of considerable importance because of its specific function in attracting a female to a male of the same species (Blair, 1958; Martof and Thompson, 1958; Littlejohn and Michaud, 1959). Along with the distinctness of the call, several other factors such as breeding seasons, breeding sites, mating behavior and temperature, and body size may prevent or inhibit interspecific matings. Several of these may operate together to provide an efficient premating isolating mechanism complex which allows the maintenance of the distinct and adapted genotype.

Recent electronical developments in sound recording and analysis permit the objective and quantitative treatment of mating calls of anurans (Blair, 1955). Thus a consideration of this apparently important isolating mechanism among the members of a closely related species group may be of interest.

Seven species of the Australian leptodactylid genus *Crinia* Tschudi appear to have the characteristics desirable for a study of the origin of differences in male call. All seven are similiar in external morphology. Nevertheless they have been separated into two groups—the *C. signifera* and *C. insignifera* superspecies —on the basis of biological criteria. This separation was made by Main (1957) for the five species then recognized. Subsequently two species were added by Littlejohn (1957, 1958). The distributions of these species fall into two definite geographically isolated regions, one in southeastern, and the other in southwestern Australia, with members of each superspecies being found in each area. The two assemblages are at present separated by an extensive arid region which reaches the southern coastline.

Because of the presumed importance of the mating call as an isolating mechanism, and of its striking divergence in these species of *Crinia,* such a component appeared to represent an important facet in the general speciation pattern of this complex (Main, Lee and Littlejohn, 1958). Accordingly male call was selected for detailed study with regard to the origin of the differences and its operation as an isolating mechanism. Thus, this isolating mechanism was investigated at four stages in the speciation process, the level being dictated by the suitability of the material:

1. Geographical variation in continuous linear distributions of a species which might exhibit clinal effects.

[1] Part of a thesis submitted for the Degree of Doctor of Philosophy at the University of Western Australia.

[2] Present address: Zoology Department, University of Melbourne, Carlton, N. 3, Victoria, Australia.

2. Divergence in disjunct allopatric populations, both within the same species and between cognate species pairs.

3. Interaction of isolating mechanisms between species which are contiguously allopatric, or have slight overlap in ranges.

4. Degree of differentiation of call under well-established sympatric conditions.

Other presumed actual or potential isolating mechanisms were also investigated to a lesser extent since complete reproductive isolation is generally held to result from the interaction of several factors.

Intrinsic isolating mechanisms operate at two levels of efficiency and may accordingly be divided into:

1. *Premating isolating mechanisms:* which include ecological; seasonal or temporal; sexual, psychological or ethological; and mechanical isolation.

2. *Postmating isolating mechanisms:* which include gametic or gametophytic isolation; hybrid inviability; hybrid sterility; and hybrid breakdown (using the terminology of Dobzhansky, 1951, page 181).

Those mechanisms listed in the first category equate to the genetic "Bars to Crossing" of Muller (1942). In the present context mating is defined as the synchronous liberation of gametes, or the effects of amplexus such that the gametes are no longer available for a second attempt. Mechanisms operating at the premating level are conservative of gametes and hence more efficient than the postmating mechanisms, even though the result may be the same in both cases. Mayr (1948) has discussed the merits and disadvantages of classifications on this basis. However, in anurans, such a distinction is workable.

Relationships

The *C. signifera* superspecies is characterized by species which are associated with permanent water and which breed throughout the year. Two species are included: *C. signifera* Girard in the east, and *C. glauerti* Loveridge in the southwest. In contrast, members of the *C. insignifera* superspecies breed in temporary ponds during the winter (late April to early September). The remaining five species are included in this category: *C. parinsignifera* Main and *C. sloanei* Littlejohn in southeastern Australia; and *C. insignifera* Moore, *C. pseudinsignifera* Main, and *C. subinsignifera* Littlejohn in southwestern Australia.

All seven species are similar in size and general morphology. Except for *C. sloanei* they exhibit polymorphism of back pattern, the same morphs being present in all six species. In some cases both living and preserved material can be identified through a consideration of pigmentation of the throats of the males and the bellies of the females (Main, 1957; Littlejohn, 1958).

The following relationships were suggested by Main *et al.* (1958) using biological criteria:

1. *C. signifera* and *C. glauerti* represent disjunct allopatric cognate species, having diverged following fragmentation of a once continuous southern distribution. It was suggested that the geographical isolation came about through climatic deterioration towards increasing aridity following a pluvial of the Pleistocene and probably during Würm time.

2. The four polymorphic species of the *C. insignifera* superspecies appear closely related. In the absence of easily detected geographical barriers which would allow speciation to proceed in the southwest, the three species *C. pseudinsignifera, C. insignifera,* and *C. subinsignifera* were considered to have been derived from a common southeastern ancestor, represented today by *C. parinsignifera.* Three successive westward invasions were postulated, each occurring during a pluvial phase of the Pleistocene. Each invader was separated from the southeast by drier interpluvial conditions.

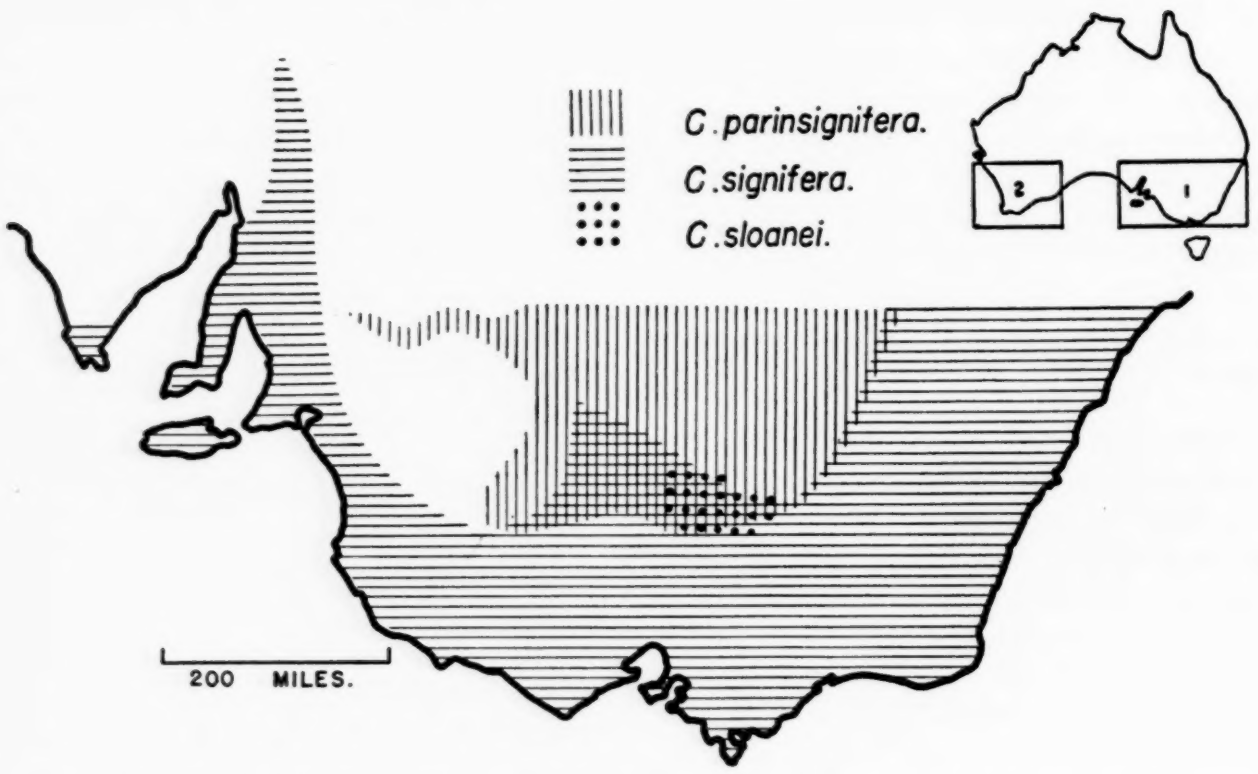


FIG. 1. Known distributions of southeastern species. The insert shows the approximate coverage of the detailed areas presented in figures 1 and 2.

On the basis of greatest difference in male call when compared to the presumed common ancestor (*C. parinsignifera*), *C. pseudinsignifera* was considered the earliest derivative, *C. insignifera* the second, and *C. subinsignifera* the third (and most recent). On the basis of the call structure (particularly pulse repetition frequency) and breeding behavior, *C. sloanei* was included within the *C. insignifera* superspecies (Littlejohn, 1958).

C. signifera and *C. glauerti* are at present isolated by an extensive arid area. *C. parinsignifera* is similarly isolated from the three related southwestern species. *C. sloanei* has a restricted southeastern distribution. Within the southeast, *C. signifera, C. parinsignifera* and *C. sloanei* are broadly sympatric. In the southwest *C. glauerti* is sympatric with *C. pseudinsignifera, C. insignifera* and *C. subinsignifera. C. pseudinsignifera* and *C. insignifera* are mainly allopatric with contiguous ranges and some areas of overlap. *C. insignifera* and *C. subinsignifera* are allopatric, being isolated by a region of prime eucalypt forests. *C. pseudinsignifera* and *C. subinsignifera* are broadly sympatric. Known distributions, believed to be complete for the southwestern species, are shown in figures 1 and 2.

MATERIALS AND METHODS

Call recordings were obtained under natural conditions at the breeding sites using a portable tape recorder (built to specifications); a battery operated preamplifier; and a Philips EL 6030 Hypercardioid microphone with 150′ of cable. Air and water temperatures were measured close to the interface, and along with other relevant data noted and included on the tape with the call recordings.

Sound analysis was carried out using a Philips GM 3156 cathode ray oscilloscope with an input signal from playback over a Pyrox TR-ST1 tape recorder. The resulting displays were photographed with a General Radio Co. Type 651-AE or Southern Instruments Minirack Type M-726, 35 mm. continuously recording camera. A neon tube activated by a 50 cycles per second alternating current was included in the camera field for time marking purposes.

Some calls were analyzed, using Car-

bonyl Iron, Type E, by the Ferrogram method of Frings and Frings (1956). These two methods gave consistent results. Call duration, number of pulses and pulse repetition frequency were readily determined by either method. Dominant frequency (Blair, 1955) and amplitude modulation were determined by cathode ray oscilloscope analysis. Call repetition rate was determined by measuring with a stopwatch the time taken for a frog to make ten successive calls in a sequence. Timing was commenced at the end of a call, then the next ten calls were measured, with complete call cycles being obtained. The dominant frequency of the calls was not subjected to detailed analysis. This component had a value in the vicinity of 2,800 cycles per second at 10.0° C. for all species.

Sound spectrograms of representative calls of each species were also prepared using a Kay Sona-Graph.

Except for the situations noted in the results, individual males produce a sequence of almost identical calls, and in most cases only one call was analyzed. Where more were analyzed a mean value was used in computation.

Temperature Correction

Since anuran call characteristics may be temperature dependent (Blair, 1955; Bellis, 1957; Thompson and Martof, 1957) it was considered necessary to correct all recorded data to a common temperature before comparison could be made between samples recorded at different effective temperatures. The data for each call component were regressed as dependent on temperature by the method of least squares. A correction factor was determined from the slope of the line, i.e. the regression coefficient. Where sufficient data were available all call components for each species were corrected before

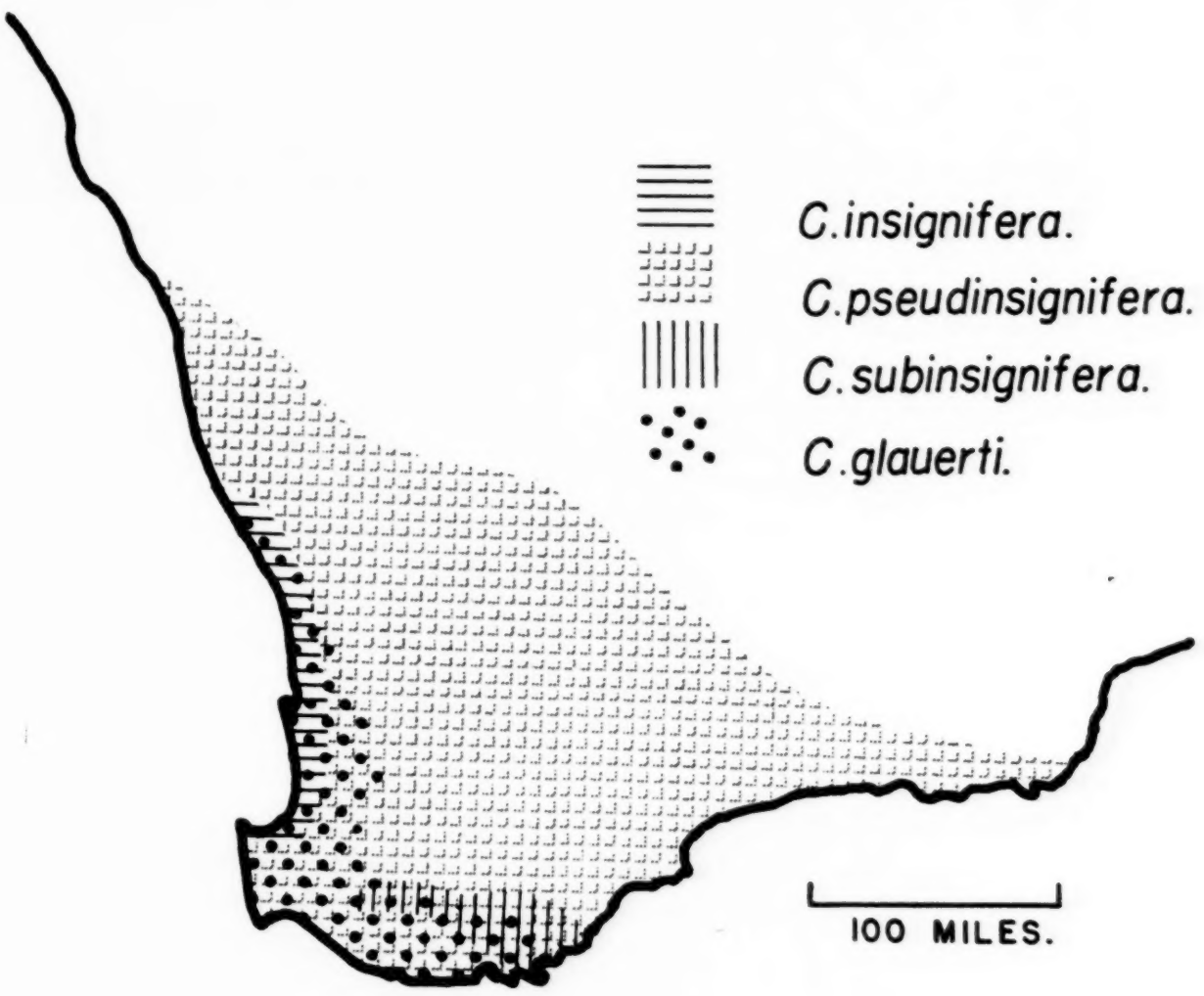


Fig. 2. Distributions of southwestern species.

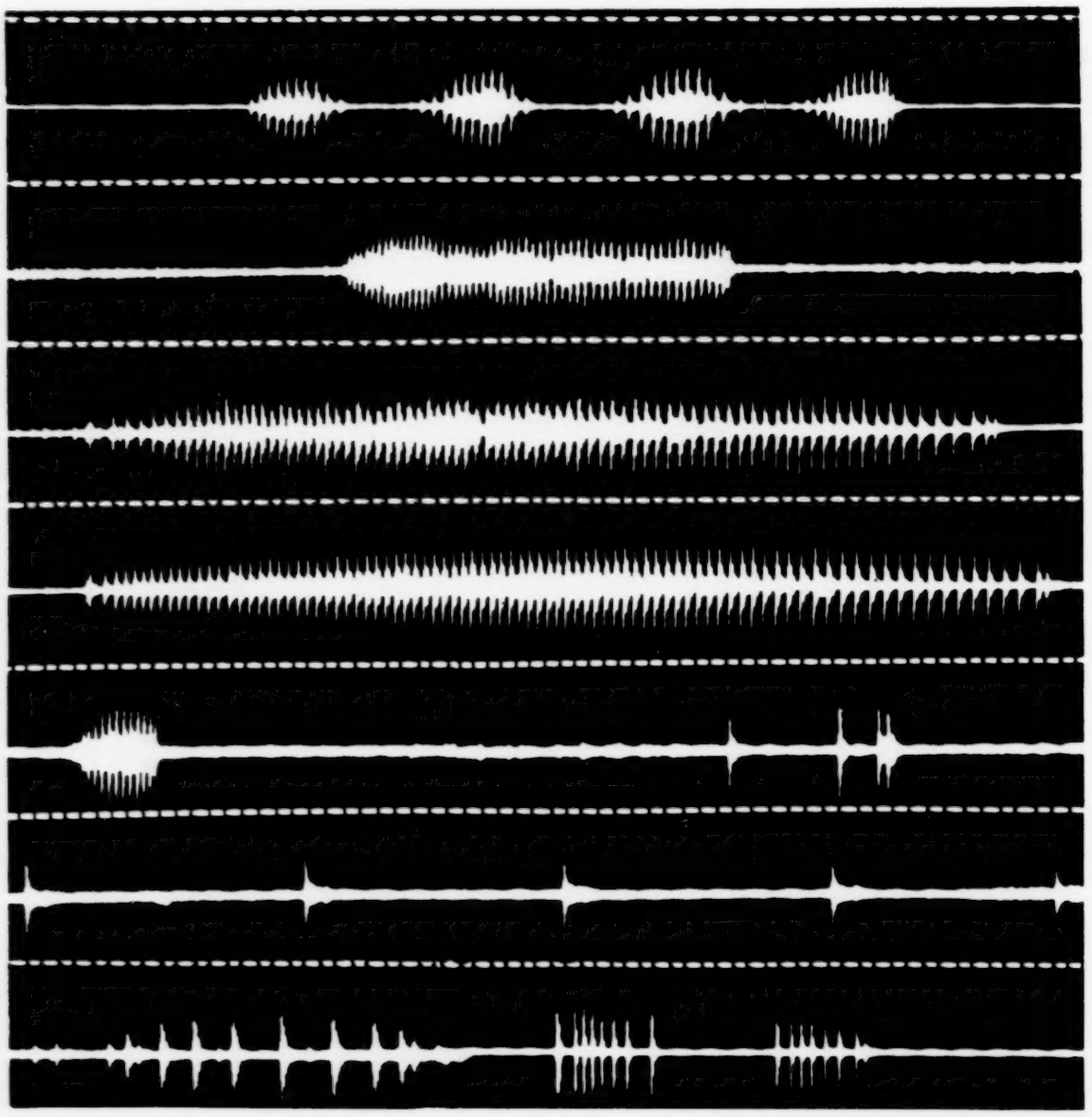


FIG. 3. Oscillograms of calls. The time marking at the top of each tracing indicates 0.01 second intervals. a. *C. pseudinsignifera;* b. *C. insignifera;* c. *C. subinsignifera;* d. *C. parinsignifera;* e. Left: *C. sloanei;* Right: *C. signifera;* f. *C. glauerti* (part only); g. Compressed pulses of: left: *C. signifera;* right: *C. glauerti.*

comparison. Common comparison temperatures were decided by those requiring minimum correction, or by converting to a temperature at which each species had been heard calling and found breeding.

Insufficient data were available for the computation of temperature corrections for *C. glauerti* and *C. sloanei.* The data for the former species were obtained from recordings made at a water temperature of 14.0° C. Recordings of *C. sloanei* calls were made at water temperatures between 10.0 and 10.5° C. Calls of *C. signifera* appeared temperature dependent only in pulse repetition frequency.

In general it may be said for the *Crinia* species that an increase in the effective temperature is accompanied by: more rapid call repetition; a decrease in call duration and in the number of pulses in a call; and an increase in pulse repetition frequency.

IN VITRO CROSSES

A number of controlled *in vitro* crosses were made following the methods of Rugh (1948). No artificial means were used to induce ovulation. Eggs of gravid females were dissected out and placed in sperm suspensions made by macerating testes in conditioned tap water. Embryos were allowed to develop at a constant temperature of either 15.5° C. or 21° C.

and experiments were continued to either hatching or metamorphosis.

Results

Call characteristics

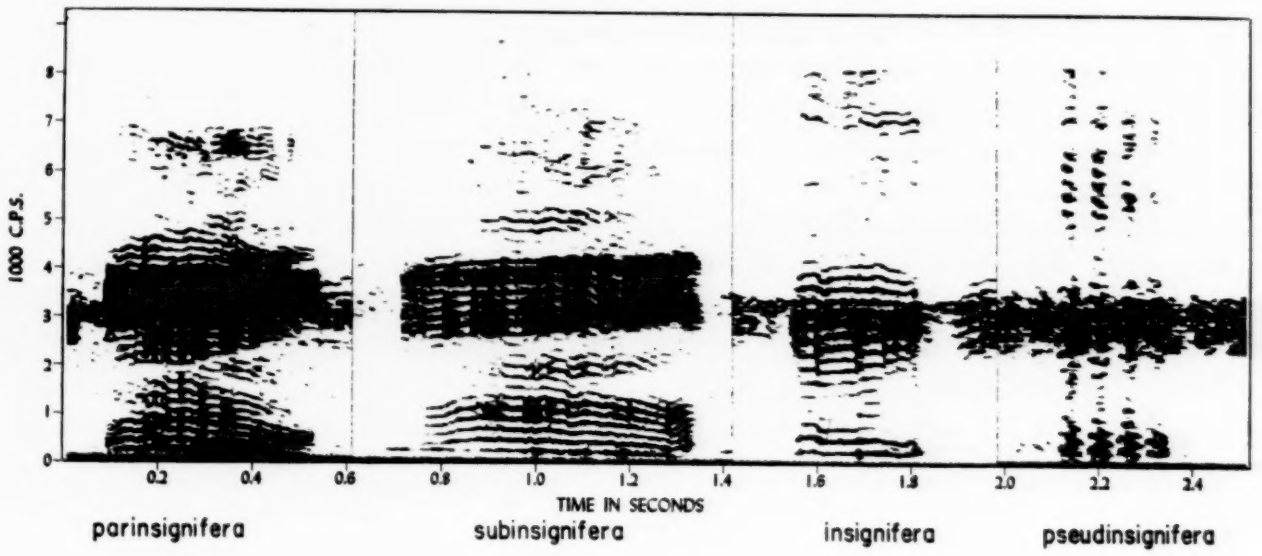
C. insignifera superspecies

Males of this group make a series of about 20–24 similar repeated calls, each composed of a group of pulses of such a frequency that the individual pulses cannot be resolved by the human ear. This pulse repetition frequency may represent the fundamental vocal cord vibration and within each pulse there is a resonant harmonic frequency of 2,000–3,500 cycles per second. No pronounced frequency modulation was evident.

The calls of *C. parinsignifera* and *C. subinsignifera* may be described as slowly repeated low pitched "buzzes"; that of *C. insignifera* as a shorter "buzz" or "peep" rapidly repeated; and that of *C. sloanei* as a short metallic "chick" also repeated rapidly. The call of *C. pseudinsignifera* is basically similar to that of *C. insignifera* but in addition there is a 3- or 4-pulsed secondary amplitude modulation so that the call sounds like a "bleet." Oscillograms and sound spectrograms of calls are presented in figures 3 and 4. Physical characteristics are given on figure 5.

C. signifera superspecies

The pulse repetition frequency of calls of these species is much lower than in the previous group. Individual pulses can



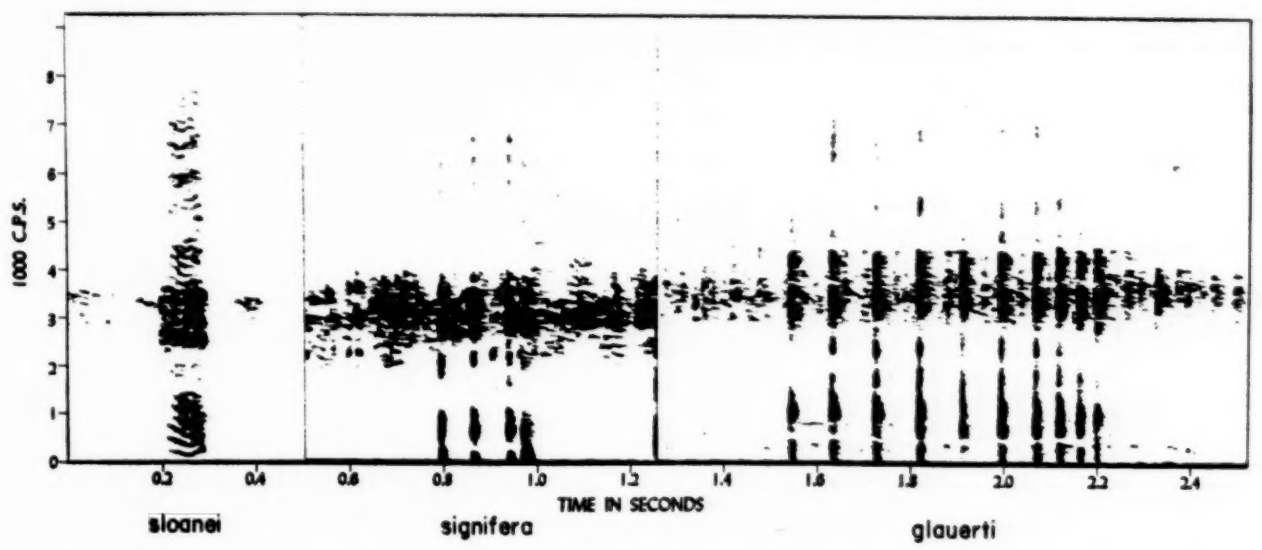


FIG. 4. Sound spectrograms of calls. Time is shown on the horizontal axis and frequency on the vertical axis. The intensity of the sound at a particular frequency is indicated by the darkness of the marking.

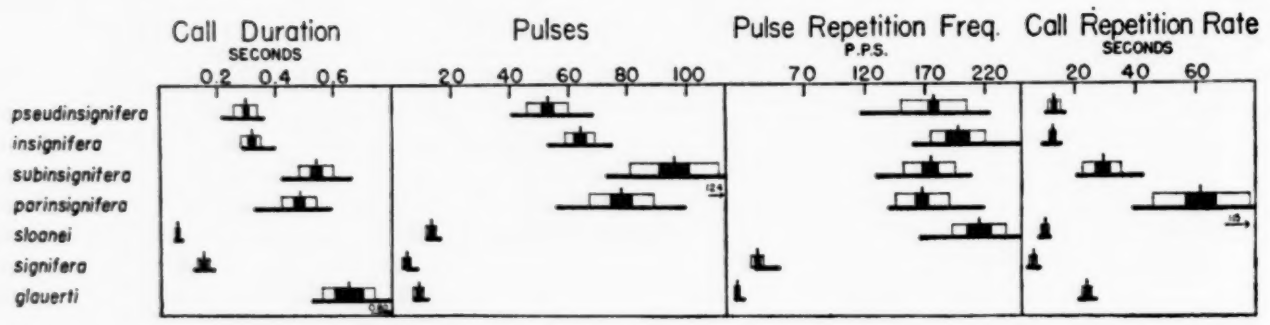


FIG. 5. Bar diagrams (after the method of Hubbs and Hubbs, 1953) for call characteristics, corrected to a common effective temperature of 10.0° C. (except *C. glauerti* which is at 14.0° C.). The heavy horizontal line indicates the range, the thin vertical line the mean; the solid rectangle includes two standard errors of the mean on either side, and the open rectangle plus half the solid rectangle indicates one standard deviation on either side of the mean. Sample sizes can also be ascertained using the techniques of Hubbs and Hubbs (1953). Data for *C. parinsignifera, C. sloanei* and *C. signifera* are from Littlejohn (1958).

be distinguished by the ear as separate "clicks" and each pulse has the appearance of a single damped oscillation. The call of *C. signifera* is composed of 4–9 pulses. Calls are repeated rapidly during a long sequence. The call of *C. glauerti* contains 7–12 pulses and is of lower pulse repetition frequency than *C. signifera.* It is also of longer duration and more slowly repeated (figs. 3, 4, and 5).

Both species exhibit occasional call variation in the form of a compressed series of pulses (fig. 3). These variations appear to occur more frequently at higher effective temperatures, particularly during the day. An individual making typical calls might suddenly produce one of the compressed type, then resume normal calling.

Call variation in continuous distributions

Sufficient data were obtained for an analysis of geographical variation in two species. In southwestern Australia one species, *C. insignifera,* has a distribution confined to the linear Swan Coastal Plain which runs along the west coast for some

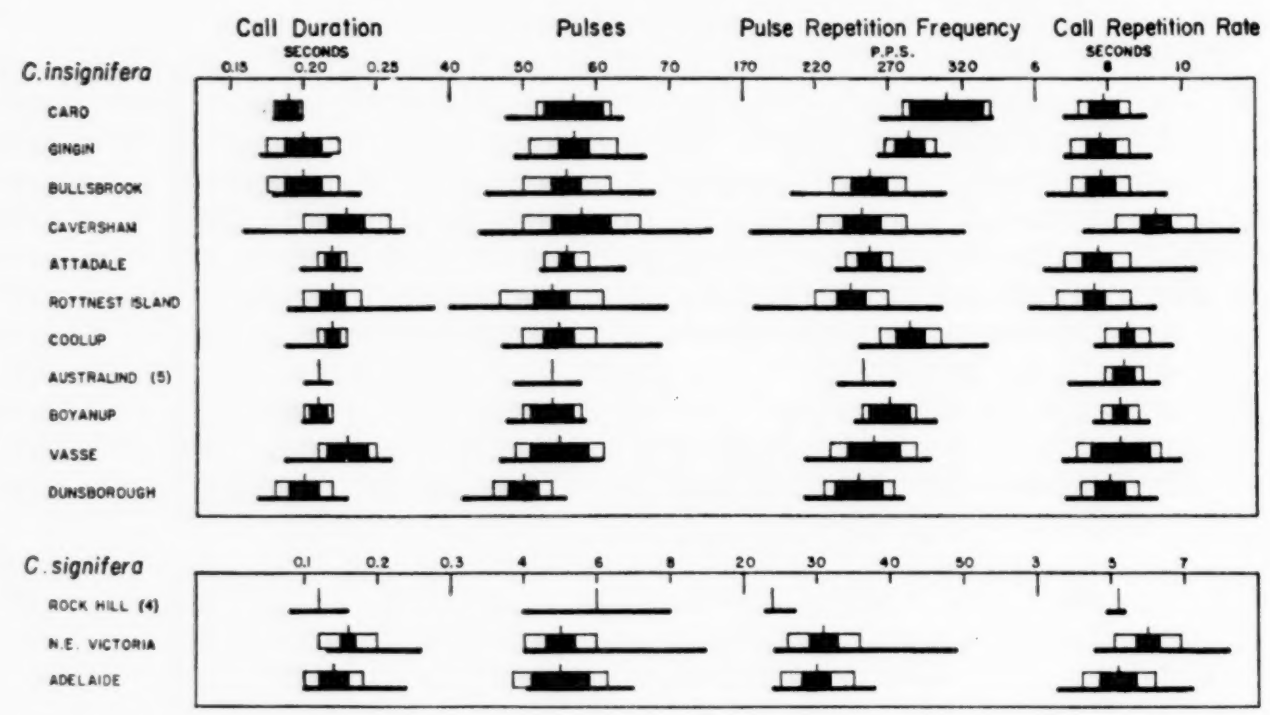


FIG. 6. Geographical variation in call structure of *C. insignifera* (corrected to 14.0° C.), and *C. signifera* (corrected to 10.0° C.). Note that data for the disjunct Rottnest Island population are also included with the mainland stations of *C. insignifera.*

300 miles, and varies in width from 20–30 miles (fig. 2). In eastern Australia *C. signifera* has an extensive and linear distribution over some 3,600 miles along the southeastern, eastern and northeastern borders of the continent.

1. *C. insignifera*

Calls of 124 individuals from eleven stations along the major dimension of the distribution were subjected to full sound analysis and an additional 121 to the determination of call repetition rate alone. The stations were distributed throughout the range of the species over approximately 240 miles from Caro in the north to Dunsborough in the south. Data were corrected to a common water temperature of 14.0° C. and are presented in figure 6. No marked or consistent clinal trend was evident for any of the four call components measured. Other aspects of the premating breeding biology also appeared geographically stable.

2. *C. signifera*

Two samples obtained for areas 500 miles apart were compared. The first sample of 27 for complete analysis, and an additional four for call repetition, was obtained by combining data from three stations: Mulwala and Tocumwal in New South Wales, and Wangaratta in Victoria. No station was more than 50 miles away from the others. The second sample of 12 for complete analysis, and an additional one for call repetition rate alone, came from Adelaide, South Australia. Only one call component, pulse repetition frequency, appeared to be temperature dependent. After correction to a water temperature of 10.0° C., the two samples were compared. Only the means of call repetition rate differed significantly ($P = 0.05$), but the ranges overlapped considerably (fig. 6).

Calls of four individuals were recorded by Dr. A. R. Main at Rock Hill in northern New South Wales, 1,100 miles from Adelaide by way of the species distribution pattern. The data for these calls fell well within the ranges of the Adelaide sample (fig. 6).

Call divergence in discontinuous geographical distributions

Three cognate population pairs appeared suitable for comparison:

1. An isolated population of *C. insignifera* on Rottnest Island, 12 miles offshore from Fremantle and the main distribution.
2. The species pair *C. subinsignifera* (southwestern Australia) and *C. parinsignifera* (southeastern Australia) (Main *et al.*, 1958).
3. The species pair: *C. glauerti* (southwestern Australia) and *C. signifera* (southeastern Australia) (Main *et al.*, 1958).

1. *C. insignifera*

Calls were recorded at three island breeding sites. Analyzed data were then corrected to an effective water temperature of 14.0° C. using correction factors determined for the adjacent mainland representatives, since water temperatures ranged over only 1.5° C. during recording on the island.

The combined island sample of calls of 32 individuals for full analysis, and an additional 16 for call repetition rate alone, was then compared with the data for the mainland representatives and found to conform (fig. 6). When the calls were compared with the nearest mainland sample, namely Attadale, no significant differences were found. No differences were evident in other aspects of breeding behavior which might act as potential isolating mechanisms. Main (1957) crossed mainland with island representatives and obtained considerable variation in the results of hybrid survival to hatching. Until further data are available, however, no conclusions can be drawn concerning hybrid capacity.

2. *C. subinsignifera* and *C. parinsignifera*

Some differentiation in call structure was evident between these two species.

The means of call duration, number of pulses and call repetition rate differed significantly. Only the means of pulse repetition frequency were not significantly different. Considerable overlap was evident in variation of call duration and number of pulses for the two species (figs. 5 and 7).

Calling seasons and temperatures overlapped, and types of breeding sites appeared similar. Some differences were evident in calling position. Males of *C. parinsignifera* called from tussocks at the centers and edges of ponds, generally 1″–4″ above the water surface. In only a few cases were males found calling while partially submerged (Littlejohn, 1958). Call characteristics were considered to be influenced by air temperatures rather than water temperatures and the former were used for temperature correction of the calls. *C. subinsignifera* males were usually observed calling while partially submerged in water, being supported by flooded vegetation. Here, water temperatures were used for correction of call data prior to comparison. While some overlap was observed, differences in male calling position might have some effect as potential isolating mechanisms.

No *in vitro* crosses were made between these two species because of lack of success in attempts to obtain gravid females of either species.

3. *C. glauerti* and *C. signifera*

In contrast to the previous condition, calls of these species were quite distinct. The degree of difference between them was of the order of the difference between the calls of sympatric species. No overlap was found for the ranges of call duration,

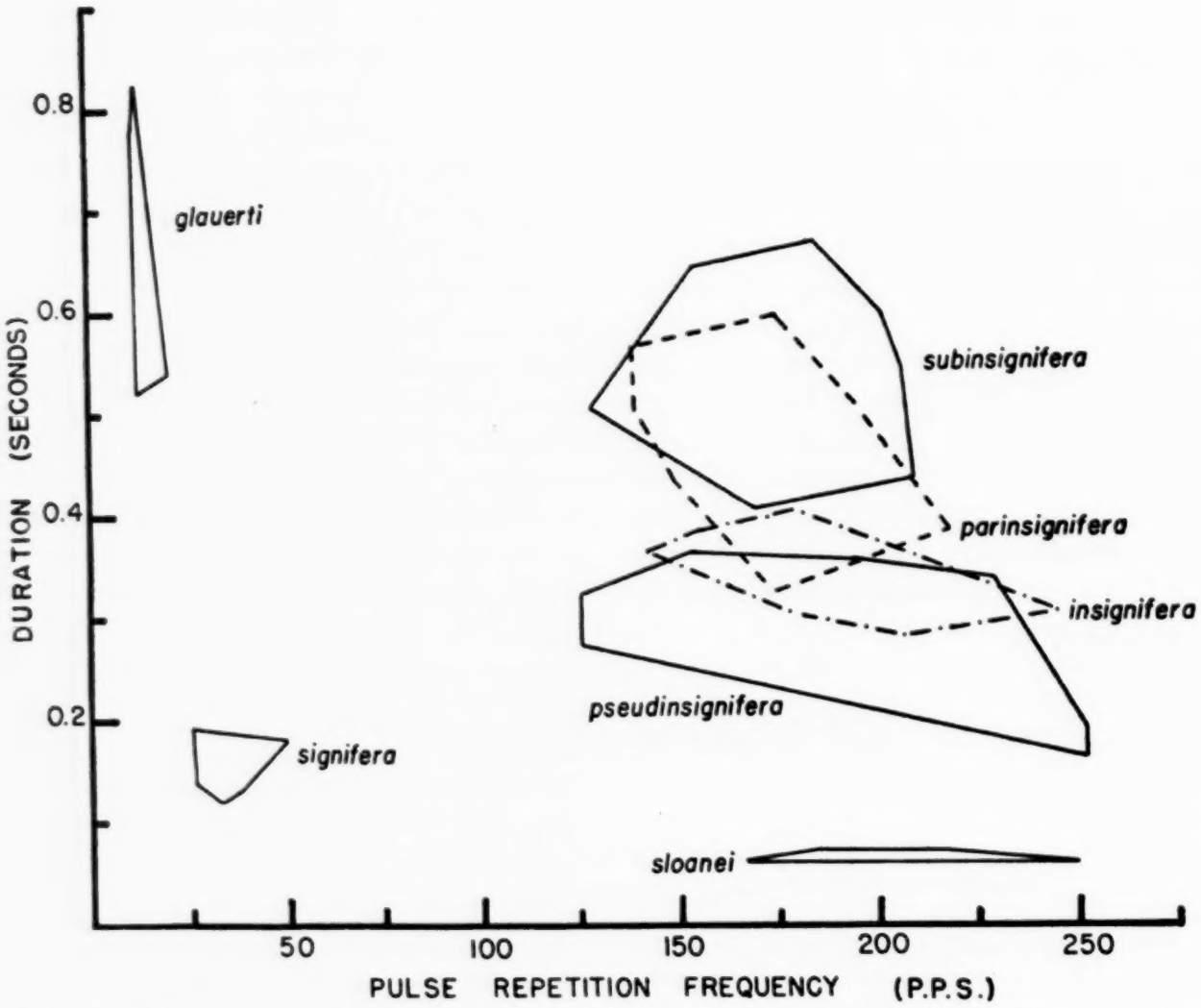


FIG. 7. Polygons enclosing the scatter of call duration against pulse repetition frequency (after the method of Blair, 1955) based on the temperature corrected data shown in figure 5. Note that the polygons of sympatric species do not overlap.

TABLE 1. *Results of crosses*

Cross number	Combination	Initial egg no.	Number hatched	Number metamorphosed	Remarks
1.	*C. glauerti* ♀ ×				
	C. glauerti ♂	12	12	7	
	C. signifera ♂	21	0	0	
2.	*C. insignifera* ♀ ×				
	C. insignifera ♂	84	85	—	
	C. pseudinsignifera ♂	86	77	—	
3.	*C. insignifera* ♀ ×				
	C. insignifera ♂	33	25	—	
	C. pseudinsignifera ♂	34	18	—	
4.	*C. insignifera* ♀ ×				
	C. insignifera ♂	71	45	—	
	C. glauerti ♂	56	25	—	
5.	*C. insignifera* ♀ ×				
	C. insignifera ♂	33	25	—	
	C. glauerti ♂	34	17	—	
6.	*C. pseudinsignifera* ♀ ×				
	C. pseudinsignifera ♂	28	20	—	
	C. subinsignifera ♂	23	8	—	
7.	*C. glauerti* ♀ ×				
	C. glauerti ♂	20	17	15	2 delayed
	C. subinsignifera ♂	23	16	4	3 delayed

pulse repetition frequency or call repetition rate. Ranges for a number of pulses ovelapped but the means differed significantly (figs. 5 and 7). Both species occupy similar ecological situations (Main, 1957), so that no potential isolation on this basis should be expected. Likewise calling seasons for both species extend over the whole year and calling temperatures overlap considerably.

One *in vitro* cross *C. glauerti* ♀ × *C. signifera* ♂ resulted in complete hybrid inviability (No. 1, table 1). Main (1957) made several crosses between these species and his results indicated that *C. signifera* ♀ × *C. glauerti* ♂ yield low hybrid survival while the reciprocal crosses give variable hybrid survival. The results indicated that a high order of genetical incompatibility is present.

Partly overlapping ranges

Investigation of geographical ranges indicated that in all but one case, namely *C. insignifera* and *C. pseudinsignifera,* the species were either disjunctly allopatric or broadly sympatric. In the latter situation reproductive isolation had apparently already developed. It was in these situations that pronounced call differences were found.

The geographical ranges of *C. insignifera* and *C. pseudinsignifera* are almost entirely allopatric, each being mutually replaced over a narrow zone marked by the Hill River, Darling and Whicher Scarps (fig. 8). Contacts of limited extent were found on 5 of 21 traverses across the replacement zone (fig. 8). Call recordings were obtained from two of these localities, namely, the Bullsbrook area, 27 miles north of Perth; and by the 163 mile post in the Vasse District, 150 miles south of Perth. The Bullsbrook sympatric region extended across the replacement for about one mile, and along it for about 1.5 miles. The Vasse region was also about one mile wide but no

assessment was made along the replacement line. Calls from allopatric choruses of *C. insignifera* and *C. pseudinsignifera* were obtained close to the Bullsbrook sympatric area, 4 miles north and 3 miles southeast respectively. Because of topographical problems, recordings of allopatric choruses in the Vasse area were obtained at Dunsborough, 10 miles north of the contact for *C. insignifera,* and at Witchcliffe, 16 miles south of the contact for *C. pseudinsignifera.* In the latter situation only three individuals were recorded but numerous typical calls were heard in the allopatric area.

Analysis of 42 calls from the two overlap areas indicated that a similar pattern of introgression was proceeding. The characteristic 3- or 4-pulsed amplitude modulation of *C. pseudinsignifera* graded through with all the intermediate types to the unmodulated call of *C. insignifera* (fig. 9). The call structure in the two species was rather similar (figs. 5 and 7). Means for number of pulses and pulse repetition frequency differed significantly but there was considerable overlap in ranges of variation. Call duration and call repetition rate did not differ significantly. The 100% amplitude modulation of the call of *C. pseudinsignifera* was the only really distinctive feature.

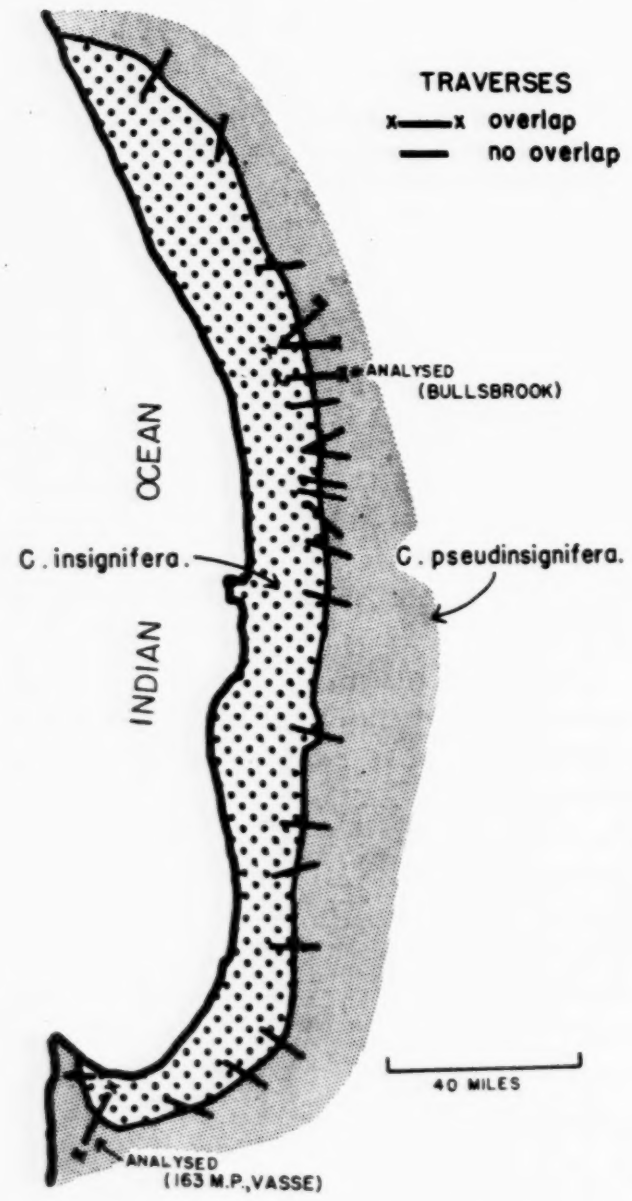


FIG. 8. Map showing the replacement of ranges of *C. insignifera* and *C. pseudinsignifera,* and traverses made across the replacement.

Since the species are on present knowledge morphologically indistinguishable, no study on this basis was possible. Types of breeding sites (shallow temporary ponds), breeding seasons, calling temperatures and calling positions are similar. Two crosses *C. insignifera* ♀ with *C. pseudinsignifera* ♂ produced a high proportion of viable hybrid larvae (Nos. 2 and 3, table 1). Main (1957) reported that two similar crosses resulted in 63% and 64% larval survival. Seven reciprocal crosses made by the same author resulted in variable larval survival ranging from 0%–91% (mean = 46%). No attempts were made to rear hybrids beyond hatching.

Sympatric species

Three broadly sympatric assemblages were present in the seven species of *Crinia* studied: one in southeastern Australia and two in southwestern Australia. Within each assemblage the species had similar or widely overlapping breeding seasons and calling-temperature ranges.

1. *C. parinsignifera, C. sloanei* and *C. signifera*

Littlejohn (1958) discussed the relationships of the three sympatric eastern species and suggested that specificity of call, and calling position constituted important premating isolating mechanisms. The calls were found to be quite distinct

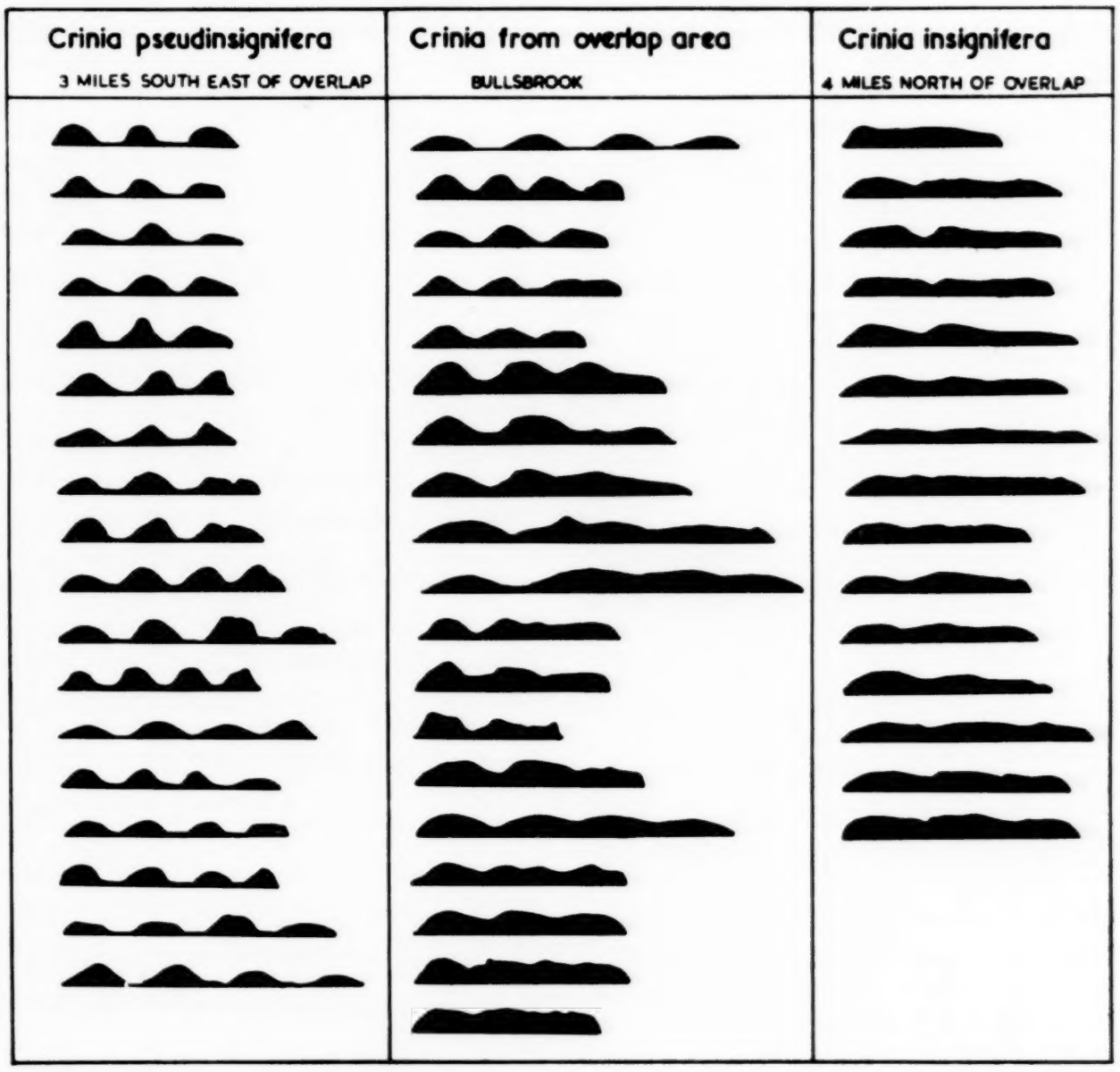


FIG. 9. Direct tracings of the upper halves of call oscillograms of *C. pseudinsignifera* and *C. insignifera* and intermediates from the Bullsbrook traverse. (The lower half is a mirror image of the upper and is not included for this reason.) Note the unpulsed calls of *C. insignifera,* pulsed calls of *C. pseudinsignifera* and the intergrading nature of the overlap sample. A similar pattern was evident in the data from the Vasse area.

(figs. 3, 4, 5, and 7), with no overlap in call duration and number of pulses for all three species. Some overlap was evident in the ranges of call repetition rate for *C. sloanei* and *C. signifera,* and pulse repetition frequency for *C. sloanei* and *C. parinsignifera,* but the means differed significantly.

All three species were frequently found calling at the same time in one pond. *C. sloanei* males were usually found calling in open water toward the center of a pond, *C. parinsignifera* males while 1″–4″ above the water surface in tussocks of partly flooded vegetation, and *C. signifera* males while partly immersed in water, supported by submerged vegetation at the edges of the ponds, often under the cover of overhanging grasses (Littlejohn, 1958).

Two crosses made under field conditions (Littlejohn, 1958) indicated reduced hybrid larval viability from the parental combination *C. sloanei* ♀ × *C. parinsignifera* ♂ with all hybrids dying before metamorphosis. Another cross by the same author—*C. sloanei* ♀ × *C. signifera* ♂—resulted in the production of transformed hybrids. Main (1957) made the cross *C. signifera* ♀ × *C. parinsignifera* ♂

and this resulted in reduced hybrid survival up to hatching.

2. *C. glauerti* and *C. insignifera*

Calls of these two species are strikingly distinct (figs. 3 and 4), with no overlap in any of the four call components measured (figs. 5 and 7). Habitat differences may assist in reducing the opportunity for these two species to cross-breed. *C. glauerti* generally occupies permanent ponds while *C. insignifera* breeds in temporary ponds. There is some overlap in transitional areas and breeding choruses of each species have been found in close proximity. Males of both species call while partly submerged in water, being supported by floating or emergent vegetation.

Two crosses *C. insignifera* ♀ × *C. glauerti* ♂ resulted in about 50% hybrid larval survival to hatching (Nos. 4 and 5, table 1). Main (1957) obtained slightly lower survivals for three crosses involving the same parental combinations. He also made five reciprocal crosses, four of which resulted in complete hybrid inviability. The other yielded larvae which hatched (19%), but all were oedematous.

3. *C. glauerti, C. pseudinsignifera* and *C. subinsignifera*

These three southwestern species appeared to overlap in all aspects of premating behavior except male call structure (figs. 3 and 5). Pulse repetition frequencies of calls of *C. subinsignifera* and *C. pseudinsignifera* did not differ significantly; and there was some overlap in call repetition rates of *C. subinsignifera* and *C. glauerti.* All of the other components did not overlap in ranges of variation.

Littlejohn (1957) found that a cross *C. pseudinsignifera* ♀ × *C. subinsignifera* ♂ resulted in high larval survival. A repeat of this cross gave a lower figure. Hybrids resulting from the cross *C. glauerti* ♀ × *C. subinsignifera* ♂ reached metamorphosis, but had truncated snouts (Nos. 6 and 7, table 1; Main, unpublished).

Discussion

From the results it can be seen that there is very little call variability over the entire geographical range of *C. insignifera,* and over 500 miles of the range of *C. signifera.* Such geographical stability seems to be a general feature of calls of all the species so far studied, at least on the basis of scattered recordings and from hearing numerous individuals through the ranges. Blair (1958) has reported that the calls of *Bufo compactilis* and *B. cognatus* are also stable over ranges of 250 and 500 miles respectively. It is only in situations where there is partial overlap in range of two species and where reinforcement is occurring that pronounced geographical variability is found (Blair, 1955, 1958).

Consideration of three pairs of cognate disjunct populations reveals a variable pattern of call divergence in allopatry, from complete absence of differentiation through to divergence as great as that found between sympatric species.

The population of *C. insignifera* on Rottnest Island has probably been isolated from the adjacent mainland representatives for about 8,000 years. At such a time the world sea level was 70 feet lower (Godwin, Suggate and Willis, 1958), and a land connection between the island and the mainland was possible. After prolonged isolation of this order, no call divergence is evident. Another leptodactylid species, *Heleioporus eyrei* (Gray), is also found on the island and the adjacent mainland. Some call recordings from each area have been analyzed, and there are indications that here also the calls have not diverged (unpublished data).

The cognate species pairs: *C. subinsignifera* and *C. parinsignifera; C. glauerti* and *C. signifera;* exhibit call divergence with significant differences being evident. In the former pair the means of three call characteristics differ significantly but

there is considerable overlap in ranges. There are also differences in male calling positions. The order of call divergence is by no means as great as that found between sympatric species. From the hybridization occurring between *C. insignifera* and *C. pseudinsignifera* it might be inferred that the call differences are at present not adequate for potential efficient premating isolation. Thus the specific status of the western isolate may be considered questionable. In the absence of hybridization and call discrimination tests, it is proposed here that, at present, the specific rank of *C. subinsignifera* should be left standing.

C. glauerti and *C. signifera* have calls which are strikingly different, being as distinct as those of sympatric species. There are also indications of considerable genetical incompatibility. It is clear that these two populations have, in allopatry, acquired all the requirements for the efficient maintenance of genetic distinctness should sympatry come about.

Call divergence in allopatry can be explained by either of two processes:

1. Incidental divergence along with general adaptive evolution of the geographically isolated populations (Muller, 1940, 1942).

2. Through the direct effect of natural selection. Each isolate might be exposed to a different "sound environment" at its breeding site. The presence of other species with similar sounding calls might induce call differentiation through selection for a distinct call and the resulting reproductive efficiency. The mechanism would be the same as that operating during reinforcement of isolating mechanisms (Blair, 1955, 1958). In this case the isolating mechanisms would evolve in the manner proposed by Dobzhansky (1941), and Muller (1942, p. 104).

The sound environments of the island and mainland populations of *C. insignifera* are relatively simple. The island population occupies the breeding site exclusively during its breeding season. The mainland population is occasionally found occupying the same sites as *C. glauerti* but the calls are in no way similar. In most cases *C. insignifera* on the mainland is also the exclusive occupant of the breeding sites. In this situation, with both isolates occupying simple "sound environments," there is no call divergence even after prolonged geographical isolation.

It cannot be stated with certainty whether the divergence in call between the members of each of the species pairs has resulted from incidental evolution or from the direct effect of selection for call distinctness. With regard to the latter possibility it is of interest to note that the call of *C. subinsignifera* is less like that of *C. pseudinsignifera,* with which it is sympatric, in call duration and number of pulses, than is the allopatric *C. parinsignifera.* This relationship does not hold, however, for call repetition rate. Thus the possibility of reinforcement, while unlikely, cannot entirely be overlooked.

If the parental stock of *C. glauerti* had a call which was similar to the present *C. signifera* then a four-pulsed call, even while differing in basic physical structure, may not have been efficiently resolved from the three to four pulsed call of the now broadly sympatric *C. pseudinsignifera* (figs. 3 and 4). Reinforcement could have led to call divergence in the invading species to result in the present call of *C. glauerti,* through the incorporation of additional pulses and a slowing of the call repetition.

Main *et al.* (1958) have postulated that *C. glauerti* and *C. subinsignifera* represent contemporary invaders into southwestern Australia. In view of the marked difference in degrees of call divergence in these species and in the possibility of there not having been any reinforcement activity, the Bassian *C. glauerti,* rather than being contemporary with *C. subinsignifera,* might represent an earlier, perhaps penultimate invader. This population could then have been isolated from *C. signifera* for a much greater length of

time, perhaps since the breakdown of a pluvial of Riss time toward arid conditions. These authors accounted for the presence of the second Bassian species, *Heleioporus inornatus,* in southwestern Australia by assuming it to represent an earlier invader contemporaneous with the second Eyrean migrants. While other presumed Bassian elements became extinct during the ensuing arid interpluvial period, this form was believed to have persisted because of its association with small peat bogs in poorly drained areas having large water catchments. If *C. glauerti* represents an earlier invader, then it must be postulated that the conditions which operated during the last pluvial phase were not moist enough to allow a small species associated with permanent water to reach the southwest.

With this alternative hypothesis, levels of call divergence could be equated directly to the length of time in geographical isolation. Such a relationship for allopatric populations has been suggested by Blair (1958), and Main *et al.* (1958).

All that can be said, after a consideration of the allopatric cognate populations, is that where divergence of call is present it can be explained either on the basis of incidental evolution over different periods of time in geographical isolation, or as a result of direct selection for call distinctness in differing sound environments. It is evident that a clearer view of the process will be gained only from the consideration of isolated populations occupying similar sound environments. For only here will the effects of incidental divergence of call be surely detected. As Dobzhansky (1951) has suggested, island populations seem most suitable. The potential of such situations is evident in the results of the comparison of the island and mainland populations of *C. insignifera.*

Whichever mechanism operated to induce call divergence during allopatry, it is clear that the two species pairs have differentiated in this characteristic, being in one case well on the way, and in the other case clearly already at the level of biological species, without any requirement for direct reinforcement between the two populations under consideration.

The geographical stability in the continuous distributions, and the lack of divergence in the island isolate of *C. insignifera* point toward incidental divergence of call as being slow and probably unimportant in accounting for all of the differences present between the calls of related sympatric species. While some incidental evolution can occur, the direct influence of selection surely adds to the causes of call evolution. The incidental divergence might set the direction in which direct selection will operate (Dobzhansky, 1951).

The consideration of the two species with partially overlapping ranges adds little constructive information to the concepts of evolution of reproductive isolating mechanisms. From the introgression occurring between *C. insignifera* and *C. pseudinsignifera* it is suggested that these populations have never previously been sympatric. Because of the limited nature of the areas in which the introgression is occurring, it appears that either the zones are stable, being maintained by environmental factors as yet undetermined, or that contact is recent following a general climatic trend toward aridity. Such a trend could lead to a westward extension of the range of *C. pseudinsignifera* through a thinning of the prime eucalypt forests which occur along the replacement. Recent activity by man in clearing the forest and in excavating may have accelerated the process.

Since all shades of intermediate types of calls are found in the overlap area, it is suggested that this call character of amplitude modulation is inherited in a polygenic manner. It also appears that the hybrids are fertile and are backcrossing. In the light of such hybridization the specific status of *C. pseudinsignifera* is questionable. Since the areas of introgression are limited in extent, it is pro-

posed for the present to leave the taxonomic position as such in accordance with the suggestions of Blair *et al.* (1957).

In the three cases of sympatry investigated, the notable feature within any group is the distinctness of the male call. There is in every instance no overlap in range of variation of at least one call characteristic, usually call duration, or pulse repetition frequency or both (figs. 5 and 7). It may be inferred that the characteristic differing most markedly, particularly in failure of overlap of variation, with that of the other sympatric species, is the critical factor for female discrimination. Most of the other components of breeding behavior, except perhaps calling position in some cases, which could operate as effective premating isolating mechanisms, overlap in their effective limits. Some of the situations appear to be backed by hybrid inviability but no consistent pattern has been seen in the interpretation of the *in vitro* cross results. Further work on hybrid cytology and the rearing of hybrids to metamorphosis or maturity may clarify the situation.

Acknowledgments

This study was carried out with the aid of a Research Grant from the University of Western Australia.

The author is sincerely grateful to Dr. A. R. Main for his continued guidance during the course of the research. The assistance of Patricia G. Littlejohn through much of the field work is gratefully acknowledged. The writer is indebted to Dr. W. Frank Blair for helpful suggestions and for reading the manuscript. Dr. Clark Hubbs also gave valuable advice.

Mr. K. W. Taplin of the Department of Electrical Engineering at the University of Western Australia made available one of the oscilloscope cameras. Mr. J. V. Fall of the same department gave advice on electronical problems. Dr. W. Frank Blair made available the Kay Sona-Graph for the sound spectrograph analysis.

Summary

1. The *Crinia signifera* and *C. insignifera* superspecies include seven closely related species which, while lacking much morphological variation, show marked differentiation in male mating-call structure.

2. Because differences in mating call are presumed to function as important premating isolating mechanisms in the sympatric species, this character was examined in detail with respect to the origin and operation of such differences. Other probable isolating mechanisms were considered along with mating call.

3. Calls were analyzed objectively by Cathode Ray Oscilloscope of Carbonyl Iron Powder, and after temperature correction, quantitative comparisons were made.

4. From an examination of material at different evolutionary levels the following suggestions are advanced for call divergence:

a. Call structure is geographically stable in continuously distributed populations of a species except under conditions of reinforcement.

b. In allopatry call structure appears to diverge slowly, with slight but significant quantitative differences present between cognate species which are presumed to have been isolated since Würm time.

c. Prolonged isolation in allopatry could lead to the establishment of call differences as great as those found between sympatric species; i.e. adequate for the efficient maintenance of reproductive isolation. Whether the marked call differences which exist between *C. glauerti* and *C. signifera* arose as a byproduct of other adaptive evolutionary processes or whether they resulted from the direct action of selection for distinctness in differing "sound environments" cannot at present be stated. They may have resulted from the action of either or both processes.

d. The advantages of studying call evo-

lution in isolated populations with simple "sound environments" is stressed.

5. In the one case of narrow overlapping range examined, introgression, as indicated by abrupt intergradation in call structure, was observed.

6. In extensive sympatric situations, calls of each species are well differentiated, with no overlap in ranges of variation of at least one call component, usually call duration or pulse repetition frequency. It is suggested that the component showing greatest differentiation is probably the critical factor by which female discrimination occurs.

Literature Cited

Bellis, E. D. 1957. The effects of temperature on salientian breeding calls. Copeia, **1957:** 85–89.

Blair, W. F. 1955. Mating call and stage of speciation in the *Microhyla olivacea-M. carolinensis* complex. Evolution, **9**: 469–480.

——. 1958. Mating call in the speciation of anuran amphibians. Amer. Nat., **92:** 27–51.

Blair, W. F., A. P. Blair, P. Brodkorb, F. R. Cagle, and G. A. Moore. 1957. Vertebrates of the United States. McGraw-Hill, New York.

Dobzhansky, T. 1941. Genetics and the Origin of Species. Columbia University Press, New York, Second Edition, Revised.

——. 1951. Genetics and the Origin of Species. Columbia University Press, New York, Third Edition.

Frings, H., and M. Frings. 1956. A simple method of producing visible patterns of tape recorded sounds. Nature, **178:** 328–329.

Godwin, H., R. P. Suggate, and E. H. Willis. 1958. Radiocarbon dating of the eustatic rise in ocean level. Nature, **181:** 1518–1519.

Hubbs, C. L., and C. Hubbs. 1953. An improved graphical analysis and comparison of series of samples. Syst. Zool., **2:** 49–57.

Littlejohn, M. J. 1957. A new species of frog of the genus *Crinia*. W. Austr. Nat., **6:** 18–23.

——. 1958. A new species of frog of the genus *Crinia* Tschudi from southeastern Australia. Proc. Linn. Soc. New South Wales, **83:** 222–226.

Littlejohn, M. J., and Ted C. Michaud. 1959. Mating call discrimination by females of Strecker's Chorus Frog (*Pseudacris streckeri*). Texas J. Sci., **11:** 86–92.

Main, A. R. 1957. Studies in Australian Amphibia 1. The genus *Crinia* Tschudi in southwestern Australia and some species from southeastern Australia. Austr. J. Zool., **5:** 30–55.

Main, A. R., A. K. Lee, and M. J. Littlejohn. 1958. Evolution in three genera of Australian frogs. Evolution, **12:** 224–233.

Martof, B. S., and E. F. Thompson. 1958. Reproductive behavior of the Chorus Frog, *Pseudacris nigrita*. Behaviour, **13:** 243–258.

Mayr, E. 1948. The bearings of the new systematics on the genetical problems the nature of species. Advances in Genetics, **2:** 205–237.

Muller, H. J. 1940. Bearings of the "*Drosophila*" work on systematics. In the New Systematics, edited by J. Huxley. Oxford University Press, Oxford.

——. 1942. Isolating mechanisms, evolution and temperature. Biol. Symp., **6:** 71–125.

Rugh, R. 1948. Experimental Embryology. Burgess, Minneapolis. Revised Edition.

Thompson, E. F., and B. S. Martof. 1957. A comparison of the physical characteristics of frog calls (*Pseudacris*). Physiol. Zool., **30:** 328–341.

ECOTYPIC VARIATION IN *NIGELLA ARVENSIS* L.

YOAV WAISEL

Department of Botany, The Hebrew University of Jerusalem, Israel

Received January 8, 1959

Nigella is a Mediterranean plant genus of the Ranunculaceae, the buttercup family. The genus consists of about 16 species of annuals. The widest distributed species is *N. arvensis* L., a polytypic group exhibiting great morphological and ecological variability. This species is found around the Mediterranean, in western Asia as well as in southern and central Europe. In Europe this species is primarily found on cultivated land, suggesting a late penetration into the region. *Nigella arvensis* and each of eight other species of the genus have, according to Pereira (1942), 12 somatic chromosomes, a count confirmed by us.

Zohary (1958) states that the Israeli forms of this group should be classified as subspecies instead of the formerly described species, *N. divaricata* Beaupré and *N. tuberculata* Griseb. Varieties of these two subspecies, namely *N. arvensis* ssp. *divaricata* var. *palaestina* and *N. arvensis* ssp. *tuberculata* var. *mutica* (fig. 1), are the subject of the present investigation.

The two taxa considered occur in the light soil belt of the Coastal Plain of Israel. The high coast line exposed to the sea wind and spray is occupied by ssp. *divaricata,* while ssp. *tuberculata* grows further inland on the sandy loam plains at low altitudes (fig. 2). These two habitats, although geographically rather close, provide striking ecological differences, and thus harbor several ecosystematic races of the local flora.

FIELD OBSERVATIONS AND EXPERIMENTS

In a transect made at the latitude of Natanya, pure populations of ssp. *divaricata* were found along the high coastal cliffs within a narrow belt of only 50–100 meters. At Beit-Lid, three kilometers from the coast, populations of ssp. *tuberculata* were located. The two taxa are spatially not completely separated, since clinal variations for several characters were found between these two sites (fig. 3).

However, the narrowness of the shore belt implied that ssp. *divaricata* might be a coastal modification of ssp. *tuberculata* (cf. Clements, Martin and Long, 1950), rather than an ecotype limited to a belt 50–100 meters wide. It appeared therefore of interest to investigate the genetic nature of the two forms.

With this aim, seeds and herbarium specimens were collected in stands of the two subspecies. The seeds were subsequently sown in an experimental plot in Jerusalem. The progenies of both, ssp. *divaricata* and ssp. *tuberculata,* showed a very close similarity to their parental forms, thus proving that the two taxa were genotypically distinct.

The occurrence of intermediates between the sites of the extreme forms called for further investigation. Field population samples were taken in the Sharon Plain in three transects at the latitudes of Natanya, Herzliya and Tel Barukh (fig. 2). All transects ran from true ssp. *divaricata* populations facing the sea, across the zone of intermediate forms, to the area occupied by populations of ssp. *tuberculata.* To ensure random sampling a large number of specimens was collected at intervals of 50 meters along the transects. Out of each collection 20 specimens were drawn and examined, using the hybrid index method (Anderson, 1949). For the construction of the indices the following characters, which appeared relevant and convenient to score, were chosen:

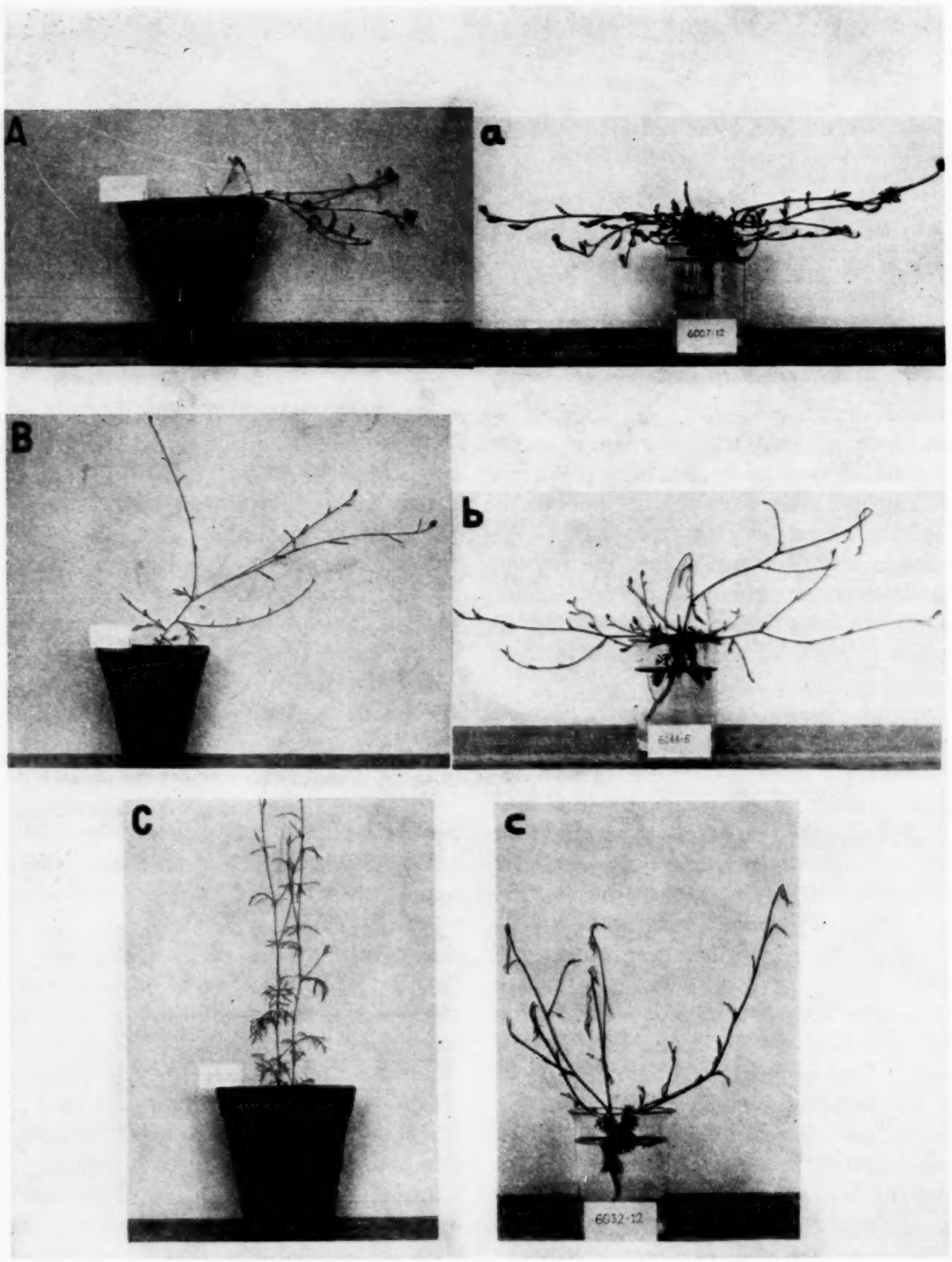


FIG. 1. A and a: *Nigella arvensis* ssp. *divaricata;* B and b: intermediate form; C and c: *Nigella arvensis* ssp. *tuberculata.* Capital letters: grown in the inland experimental plot in Jerusalem; small letters: grown in the coastal experimental plot at Mikhmoret.

1) *Plant height.* The height of the coastal type (ssp. *divaricata*) varies from 5 to 15 cm., while that of the inland type (ssp. *tuberculata*) is about 20–50 cm.

2) *Number of leaf lobes.* The leaves of the coastal type are either entire or with very few lobes, whereas those of the inland type are dissected.

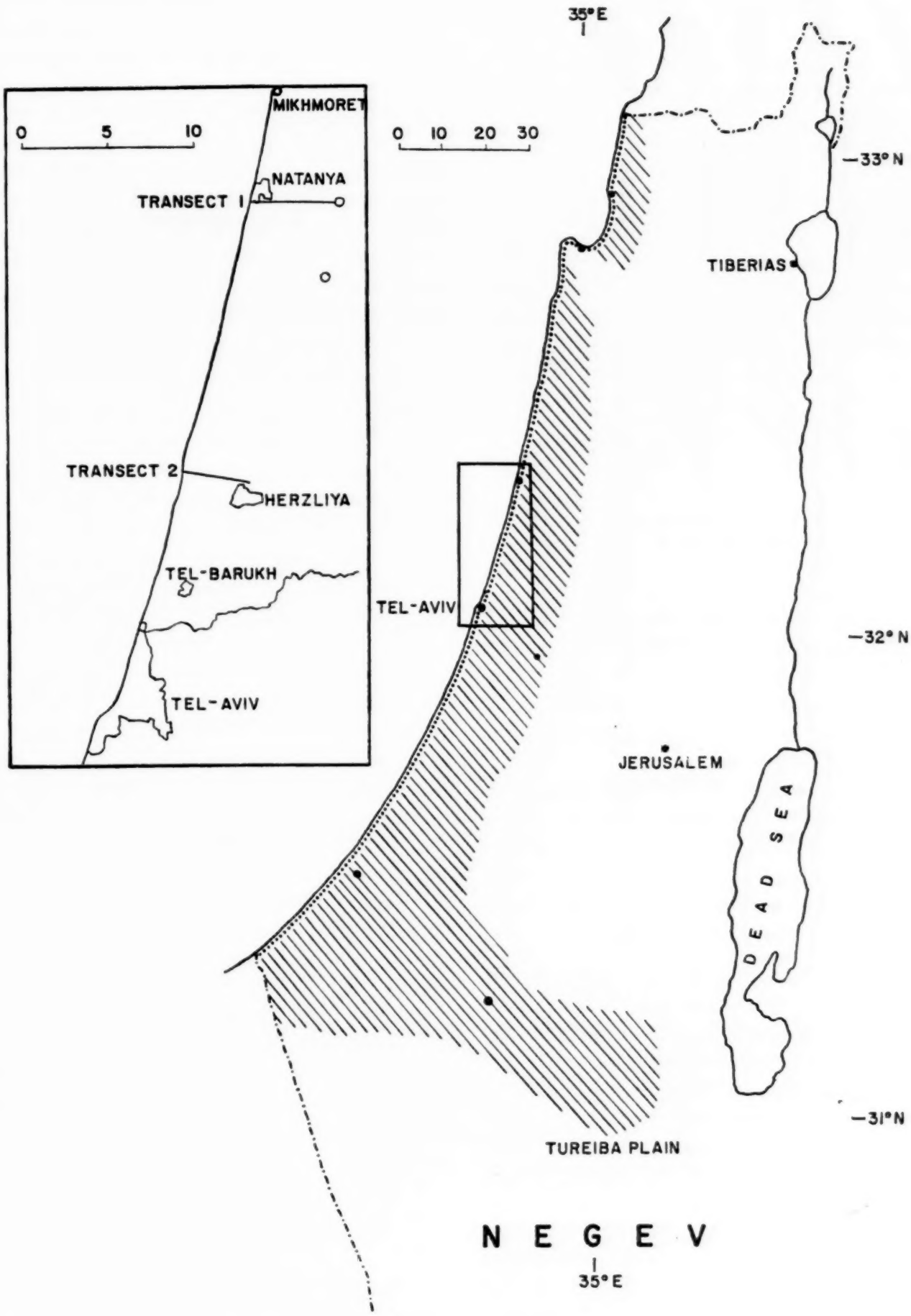


FIG. 2. Approximate distribution area of ssp. *divaricata* and the heavily introgressed intermediates (dotted), and of ssp. *tuberculata* (diagonal lines) in Israel.

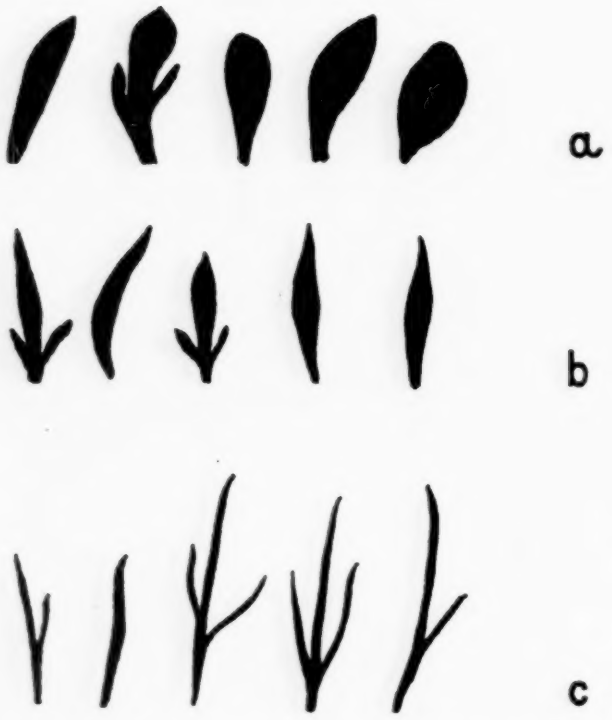


FIG. 3. Inter- and intra-population variation of leaves: a—ssp. *divaricata;* b—intermediate forms; c—ssp. *tuberculata.*

3) *The ratio of leaf length/leaf width.* Ssp. *divaricata* has oblong or elliptical leaf lobes, while the lobes of ssp. *tuberculata* are long and linear. The width of the terminal lobe was used as an indicator of the leaf width.

4) *The angle between the flower-bearing branches.* The branches of the coastal type are procumbent and divaricate at angles up to 120°. In the erect inland type the angles between the flower-bearing branches do not exceed 60°.

Characters (2) and (3) were determined on the third leaf below the flower. This leaf was found to be the most typical of the plant, and to exhibit the greatest differences between the two subspecies.

Each of the four characters was determined quantitatively and classed into four categories, to which the respective values 0–3 were assigned, 0 representing typical ssp. *divaricata* and 3 typical ssp. *tuberculata.* These values were totalled for each specimen and the individual scores plotted on a bar graph. The sum of 0 represents the pure ssp. *divaricata,* while that of 12 represents the extreme limit of ssp. *tuberculata* in all four characters. Results of illustrative examples are given in figure 4.

A further investigation revealed about 98 per cent of fertile pollen grains in all three types. Crossing and back crossing were successful.

F_1 hybrids were grown together with the parental forms in two experimental plots, one in Mikhmoret on cliffs facing the sea, and the other in Jerusalem under inland conditions. Observations on the growth and behavior of the plants were made frequently. Hybrid indices of the above forms are presented in figure 5.

RESULTS AND CONCLUSIONS

The histograms of the field samples (fig. 4) manifest the gradual transition of one ecotype into the other along the transects. This suggests that the intermediate forms could be either of hybrid nature or could represent fairly constant intermediate combinations. This suggestion is supported by comparison with the histograms of the artificial hybrids (fig. 5). Furthermore, the offspring of the intermediate plants exhibit a relatively higher variability as compared with the progenies of the two extreme types. No decrease in fertility or any barrier of this type exists between the two ecotypes, which also is demonstrated by the high fertility of the F_1 hybrids.

The histograms (fig. 4) suggest that the introgression proceeds from the coastal toward the inland type, changing the characters of the inland populations of the Sharon Plain. This introgression is most noticeable within the first few hundred meters from the coast. The most extreme populations of the inland type are found far from the coast in the central Negev sand dunes.

In spite of the presumably free gene flow from the inland plants of ssp. *tuberculata,* the populations of ssp. *divaricata* on the exposed coast remain almost pure, though in a very narrow belt. This is no doubt a result of the abrupt change there in the environment. The extreme conditions for plant growth on the coast do not permit survival of unadapted forms.

Thus, introgression in this direction is apparently prevented by natural selection.

The latter possibility was demonstrated in the experimental plot at Mikhmoret, where the two types and the hybrids were grown together. The erect inland form suffered badly from sea winds and spray (fig. 1c), all the branch tips being salt-burnt and the plants failing to produce any ripe fruit. In the hybrids, the erect branches were similarly affected (fig. 1b), but since some of the branches were prostrate a few seeds were produced. The procumbent coastal type, since it was in its natural habitat, developed and produced seeds normally (fig. 1a).

Under the extreme conditions of the coastal experimental plot, slight phenotypical changes of the inland form and of the hybrids developed. These modifications show adaptational trends toward the characters of the coastal type, i.e. towards prostrate habit, increased leaf width, etc. The latter are no doubt among the characters favored on the coast by natural selection (cf. Clausen, Keck and Hiesey, 1947, 1948). This trend is manifested in the histograms (fig. 5 b and c), which,

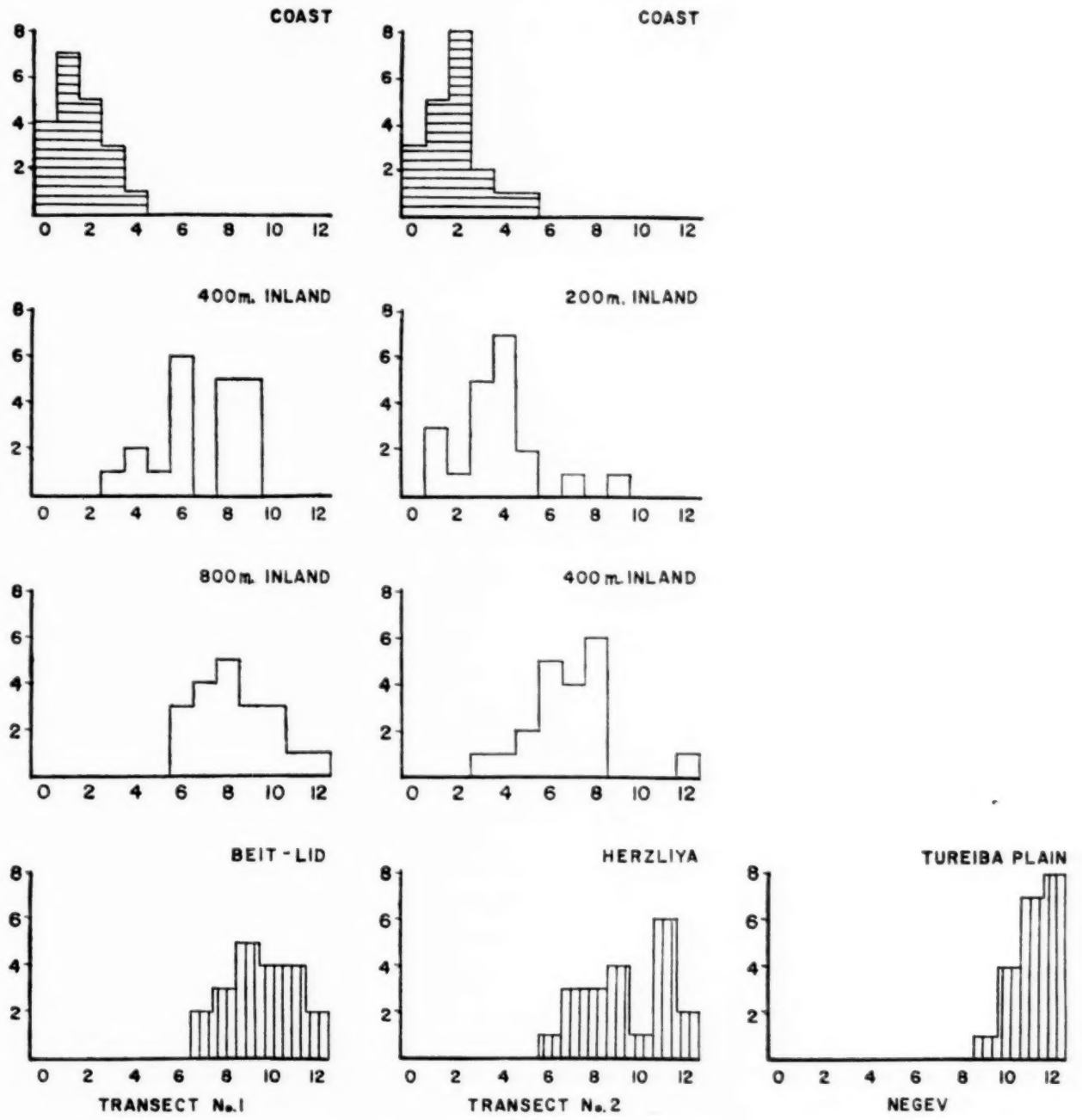


FIG. 4. Histograms of population samples collected along coast to inland transects north of Tel-Aviv (see insert map in fig. 2). Horizontal lines: ssp. *divaricata;* vertical lines: ssp. *tuberculata;* blank: intermediate populations.

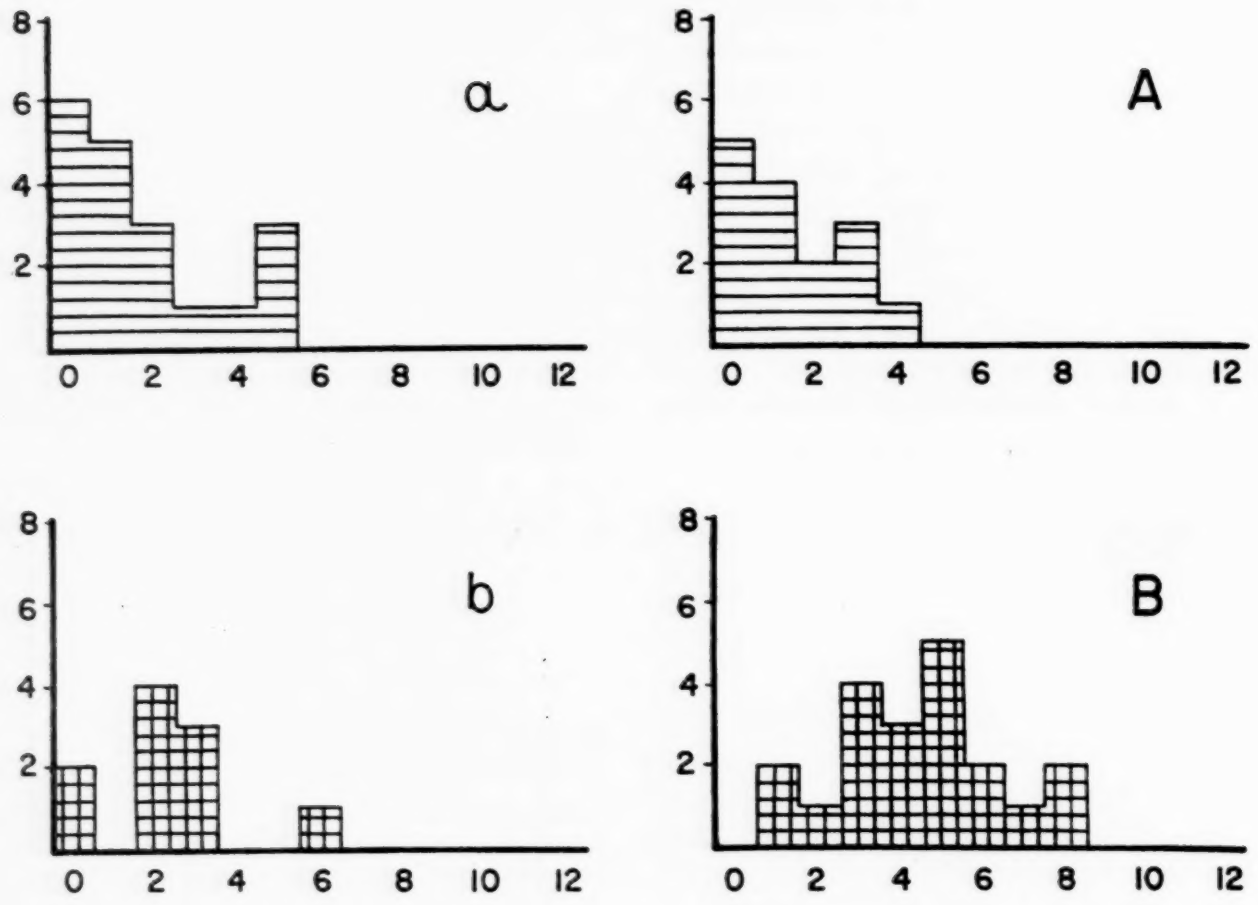


FIG. 5. Histograms for the artificial F_1 hybrids and their parents. A and a: ssp. *divaricata;* B and b: F_1 hybrids; C and c: ssp. *tuberculata.* Capital letters: grown in the inland experimental plot in Jerusalem; small letters: grown in the coastal experimental plot at Mikhmoret.

when compared with figure 5B and C, show a slight shift from the value of 12 for the true inland type, towards the value of 0 for the true coastal type.

Whereas the selective advantage of the prostrate habit under the coastal conditions has been demonstrated, the advantage, if any, of other characters, e.g. broader and less divided leaves, is not obvious, and calls for further investigation. These features are, however, characteristic of many other coastal ecotypes, just as the erect habit is characteristic of inland forms of many plant species.

SUMMARY

The relations between pure and intermediate populations of *Nigella arvensis* ssp. *tuberculata* and *N. arvensis* ssp. *divaricata,* both growing in the Coastal Plain of Israel, were investigated under field conditions and in experimental plots.

Pure populations of ssp. *divaricata* occur only within a very narrow coastal belt (50–100 meters) exposed to the sea, while pure populations of ssp. *tuberculata* are limited to inland conditions.

A gradual transition from one ecotype to the other was observed. Hybrid indices obtained from field sampling, as well as from artificial crosses between these subspecies, suggest that the intermediate forms might be of hybrid nature. Crosses and back crosses to each of the parental types gave fertile F_1 progeny.

Introgression of "coastal" characters in inland populations of the Sharon Plain was observed, but "inland" characters were not encountered in the coastal populations.

Plants of the same population, grown in two experimental plots, one under coastal and the other under inland conditions, exhibited some differences. Under the extreme conditions of the coastal plot, slight phenotypical modifications towards the coastal habit occurred both in the inland and in the intermediate forms. Under these conditions the inland plants did not produce seed.

ACKNOWLEDGMENTS

The author expresses his thanks to Prof. M. Zohary for his continuous interest. Thanks are due to Mrs. Irene Gruenberg-Fertig and to Miss Ziva Broide for reading this manuscript.

LITERATURE CITED

ANDERSON, E. 1949. Introgressive Hybridization. John Wiley & Sons, New York.

CLAUSEN, J., D. D. KECK, AND W. M. HIESEY. 1947. Heredity of geographically and ecologically isolated races. Amer. Nat., 81: 114–133.

——, —— AND ——. 1948. Experimental studies on the nature of species. III. Environmental responses of climatic races of *Achillea*. Carnegie Inst. Wash. Publ. 581.

CLEMENTS, F. E., E. V. MARTIN AND F. L. LONG. 1950. Adaptation and Origin in the Plant World. Chronica Bot., Waltham, Mass.

PEREIRA, ALICE DE LEMOS. 1942. Contribuiçaõ ao conhecimento cariológico do género *Nigella* L. Bol. Soc. Broteriana, **16:** 5–42.

ZOHARY, M. 1958. The intraspecific taxa of *Nigella arvensis* L. in Israel (Manuscript).

DISCRETE POPULATIONS DERIVED BY INTERSPECIFIC HYBRIDIZATION AND SELECTION IN *NICOTIANA*[1]

HAROLD H. SMITH AND KEVIN DALY

Brookhaven National Laboratory, Upton, L. I., N. Y.
and Department of Plant Breeding, Cornell University,
Ithaca, N. Y.

Received January 19, 1959

One approach toward understanding genetic systems that differentiate populations and their evolutionary potentialities is to produce experimental populations and to study them under conditions of controlled culture, mating, and selection. An advantage of this method over that of analyzing existing natural populations is that the variables which influence gene frequency are controlled and accessible. An obvious disadvantage is that it is hazardous to make direct analogy or to transfer unqualified interpretation to evolution in nature. In spite of this limitation, some of the most significant contributions to the study of evolution are being made by manipulating genetic systems of populations under artificial experimental conditions.

In the investigation reported here, a study was made of the effect of selection following interspecific hybridization on morphological divergence and genetic isolation at two levels of ploidy.

MATERIALS AND METHODS

Inbred strains of *Nicotiana langsdorffii* Weinm. (n = 9) and *Nicotiana* × *sanderae* Hort. var. 'Sutton's Scarlet' (n = 9) were used as parents in these experiments. Their F_1 hybrid, *N. langsdorffii* × *N. sanderae,* is vigorous, partially pollen sterile, and sets an abundance of seed. Cytological examination of meiosis shows a modal configuration of six bivalents, a quinquevalent, and a single univalent at metaphase I (Smith, 1937a; Lee, 1950). The amphidiploid, produced from colchicine treatment of germinating seedlings of the F_1 hybrid (Smith, 1943), is classified as a segmental allopolyploid and segregates in subsequent selfed generations.

The length of the corolla tube base, which extends from the insertion point of the epipetallous stamens to the base of the ovary, was chosen as a single criterion for selection (fig. 1). The conspicuous difference in length of tube base is the polygenically controlled character which best separates the parental strains (Smith, 1937b). From the progeny of the F_1 hybrid, single F_2 individuals having the smallest, intermediate, and largest corolla tube base were selected and self-pollinated. These selected lines were designated as LS_1, LS_2, and LS_3, respectively. On the amphidiploid level, similar selections were made among the progeny of the doubled F_1 hybrid and self-pollinated. These were designated as $LLSS_1$ (small), $LLSS_2$ (intermediate), and $LLSS_3$ (large). Each of these selected lines was subsequently advanced to the F_{10} generation by self-pollination and single-plant selection. The average number of individuals in the F_3 to F_{10} cultures, from each of which one plant was selected, was thirty-six.

To serve as an indication of the degree of isolation between the selected lines and their parents, studies were made of meiotic irregularities, pollen abortion, and crossability. Studies of pollen abortion were completed for both diploid and amphidiploid selected lines, while data on

[1] A portion of the research reported here was carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.

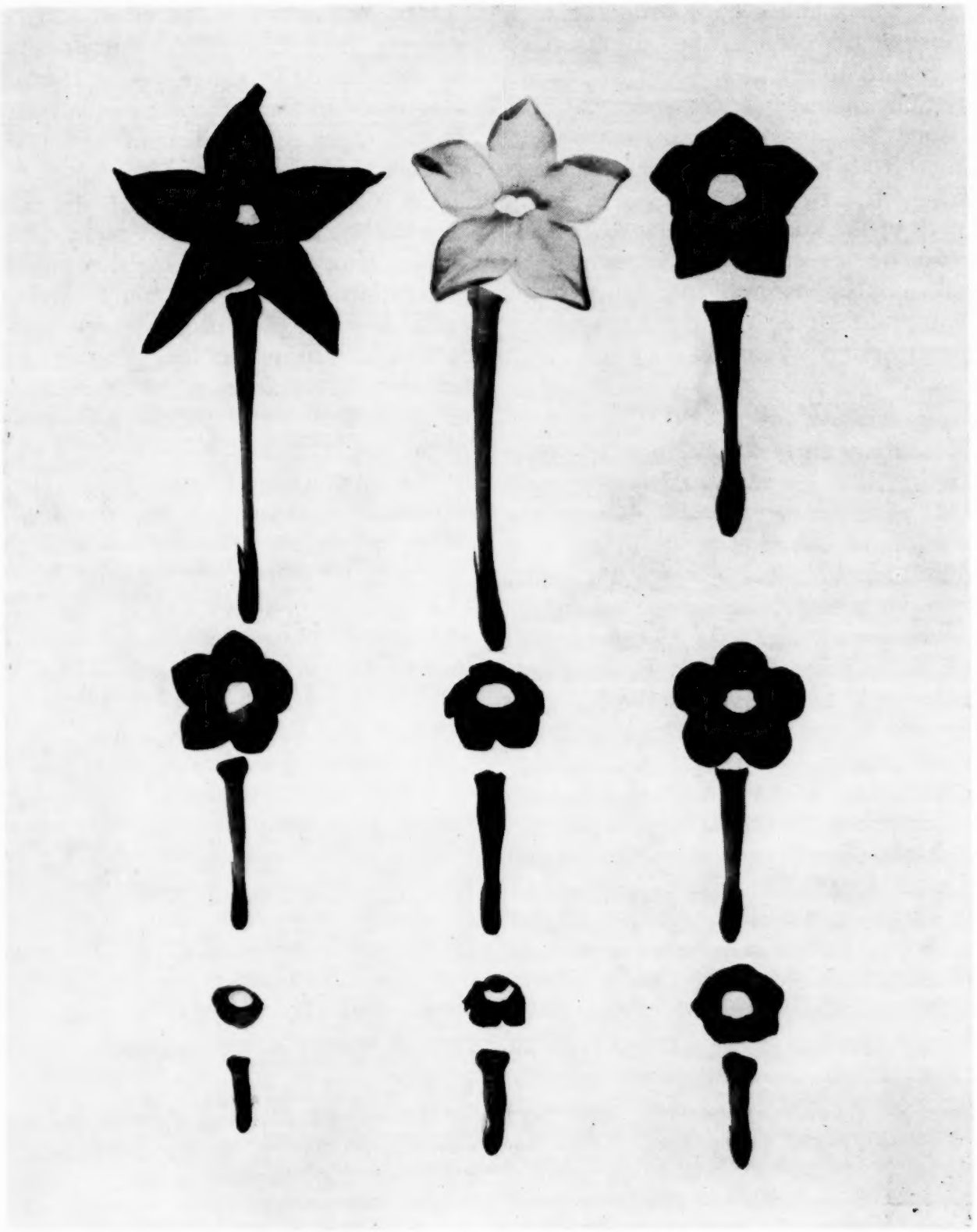


FIG. 1. Corollas of *Nicotiana* types, × 7/8. Left column: lower—*N. langsdorffii* (P_1 or LL), middle—F_1 hybrid (LS), upper—*N. sanderae* (P_2 or SS). Center column: diploid selections: lower—small (LS_1), middle—intermediate (LS_2), upper—large (LS_3). Right column: amphidiploid selections: lower—small ($LLSS_1$), middle—intermediate ($LLSS_2$), upper—large ($LLSS_3$). The selections shown are in the F_9 generation.

meiosis and crossability were collected from diploids only.

For examination of chromosome number and stages of meiosis, anthers were smeared in one per cent aceto-orcein. Determinations of pollen abortion were based upon their staining reaction with cotton blue in lactophenol. Approximately 1,000 grains were scored on slides prepared from freshly opened flowers.

Grains which appeared nonstaining and/or shrunken were classified as nonviable (cf. Smith, 1952).

For the purpose of morphological comparisons, ten unselected characters were chosen from which measurements were taken in the F_9 generation. Ten plants of each of the six selected lines were cultured in the greenhouse together with an equal number of each of the parental strains and their F_1 hybrid. A completely random design was used.

Response to Selection

Measurements of corolla tube base were made in the F_2 and each subsequent selfed generation. Twice during the course of the selection program, the diploid and amphidiploid selected lines were grown in a single experiment with the parental lines and their F_1 hybrid. In 1953, both F_3 and F_6 selections were represented, while in 1957 only the F_9 selections were included. In order to equalize the environmental variances of the parental lines, data on tube base length were transformed to the logarithmic scale base 10. Estimates of generation means and variances are presented in table 1.

In the F_3 generation, selection on the diploid (LS) level has been effective in producing lines that are clearly distinguishable as relatively small, intermediate, and large corolla types. From the F_3 to the F_9, the means of the small and large selected lines have been further shifted by selection. In the F_6 generation, the magnitude of the variances of the selected diploid lines has fallen to a level no greater than their inbred parents.

On the amphidiploid (LLSS) level, selection has also produced three distinct lines. However, in an equal number of generations as on the diploid level, the same selection pressure has been less effective in shifting the means of the small and large selections in the direction of either parent. The phenotypic variance of the amphidiploids is less in early segregating generations as shown by the smaller variances in these F_3's as compared to those derived from the F_1 hybrid. Subsequently there has been a gradual release of genetic variability which is reflected in an increase in variance of the three selected lines in the F_6 generation. The small selected line shows a further increase in the F_9 generation.

An inspection of the mean and variance of the intermediate diploid selected line reveals that it has been possible to stabilize a pure breeding, intermediate type similar in mean to the F_1, yet possessing no greater variability than either inbred parent. While the means of the amphidiploid small and large selected lines have approached the parental extremes less closely than the corresponding diploid

Table 1. *Estimates of generation means (log $\bar{x}$) and variances (s^2) for length of corolla tube base in parental species (P_1 and P_2), interspecific F_1, and selected lines originating from the hybrid F_1 (LS) or from the amphidiploid (LLSS)*

Type	Year	Small		Intermediate		Large	
		log $\bar{x}$	s^2	log $\bar{x}$	s^2	log $\bar{x}$	s^2
P_1, F_1, P_2	1953	0.6643	0.00093	1.2612	0.00086	1.7174	0.00084
P_1, F_1, P_2	1957	0.7073	0.00030	1.2896	0.00036	1.7803	0.00073
LS, F_3	1953	0.9724	0.00461	1.2586	0.00608	1.5985	0.00665
F_6	1953	0.8653	0.00088	1.3232	0.00075	1.7078	0.00081
F_9	1957	0.9440	0.00043	1.3099	0.00079	1.8016	0.00043
LLSS, F_3	1953	1.0936	0.00308	1.1687	0.00487	1.3229	0.00160
F_6	1953	0.9482	0.00347	1.2625	0.00578	1.3694	0.00461
F_9	1957	1.0052	0.01270	1.3115	0.00316	1.4873	0.00339

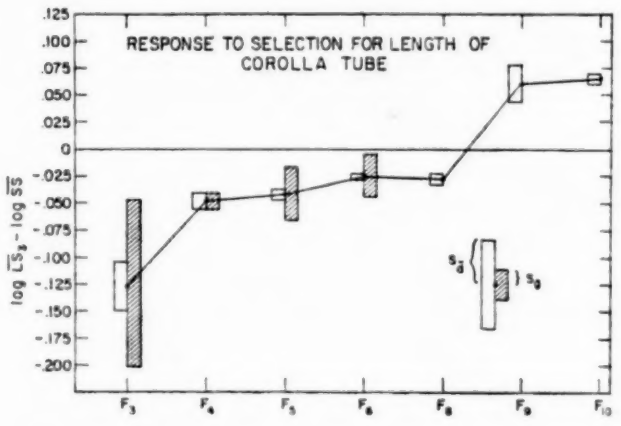


FIG. 2. Response to selection for long corolla tube in the F_3 to F_{10} generation following hybridization of *N. langsdorffii* × *N. sanderae*. In the F_6, the mean of the selected line (LS_3) is not significantly different from that of *N. sanderae* (SS); in the F_9 and F_{10}, it is significantly greater. [s_d = standard deviation of the difference between the mean of the selection (LS_3) and the mean of *N. sanderae* (SS); s_g = standard deviation based upon genetic variance of the selection (LS_3).]

selected lines, one of the latter, the large, has surpassed both the mean and the range of the original large-flowered parent, *N. sanderae* in the F_9 and F_{10} generations (fig. 2).

In the F_5 generation, the large diploid selected line (LS_3) was backcrossed to *N. sanderae* and it was noted that some of the resulting plants had flowers larger than either *N. sanderae* or the large selected line (Smith, 1954). Through

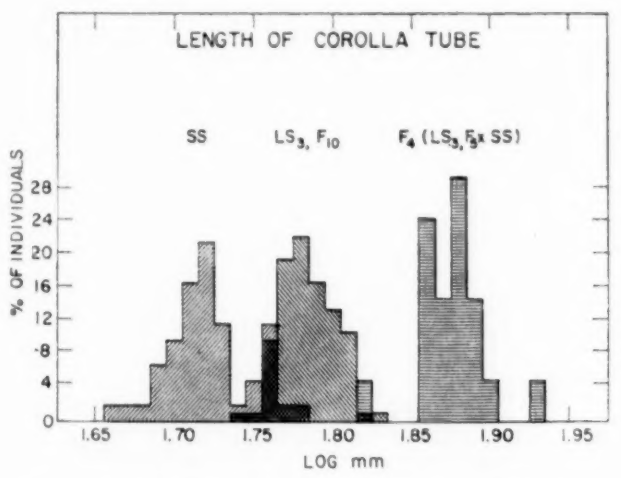


FIG. 3. Frequency distribution histograms showing the length of the corolla tube base in three populations: *N. sanderae* (SS), the F_{10} generation of the large-flowered diploid selected line (LS_3), and the F_4 generation originating from a backcross of LS_3 (then in the F_5 generation) to *N. sanderae*.

single-plant selection and self-pollination, this new line was advanced to the fourth inbred generation. In figure 3, frequency distribution histograms for corolla tube length are shown for this population compared with *N. sanderae* and the large LS_3 selected line. The new population of backcross origin forms a clearly transgressive line that exceeds in flower size any other line derived from intercrossing these species.

CYTOLOGY

Determinations of chromosome number were made at meiosis for the diploid selected lines in the F_{10} and for the amphidiploids in the F_9 generation. Counts could be made most readily from the sum of two anaphase I daughter nuclei. Complements of 2n = 18 and 36 in the diploid and amphidiploid selections, respectively, agreed with the predicted numbers based upon a somatic complement of eighteen in the original parents. The fact that these numbers have been maintained through ten generations in spite of meiotic irregularities, particularly at the amphidiploid level, indicates a high selective advantage of the balanced genome.

Irregularities of chromosome distribution were scored at anaphase I of meiosis. Aberrations consisted principally of one or two lagging chromosomes in addition to chromosome bridges and fragments. The frequency of these aberrations was expressed as a percentage of the number of cells studied and is summarized for the diploid selected lines and their parental backcrosses in table 2.

Backcrosses to *N. langsdorffii* have consistently higher frequencies of AI aberrations than the corresponding *N. sanderae* backcross of the same selection. A similar relationship was demonstrated by Smith (1952) for parental backcrosses of the original F_1 hybrid in which the percentage of pollen abortion of the *langsdorffii* backcross consistently averaged higher than the *sanderae* backcross.

If the aberration frequency in backcrosses is compared with the frequency in

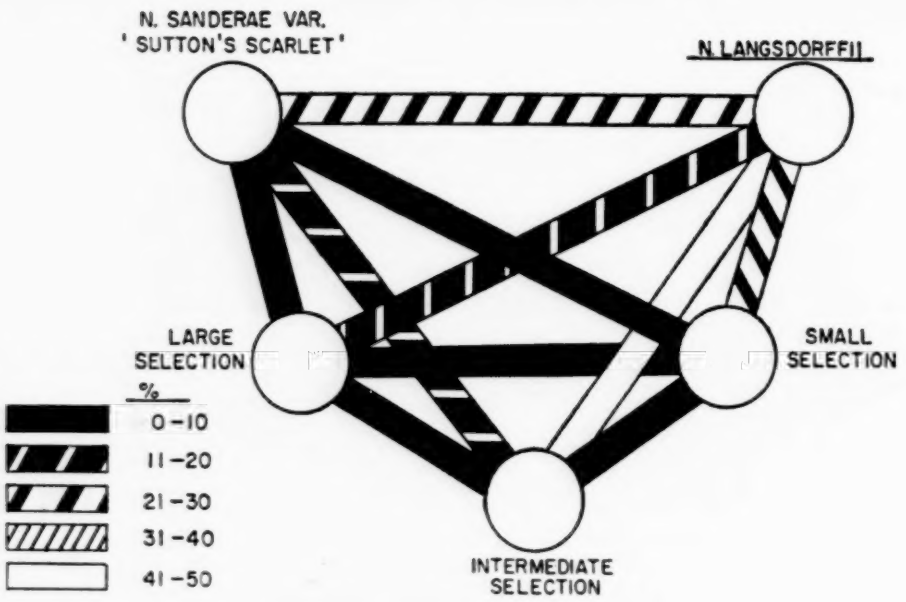


FIG. 4. Diagrammatic representations summarizing degrees of genetic isolation among diploid selected lines and between these selected lines and parental species; based on percentage of chromosomal aberration (A), percentage of pollen abortion (B), and crossability (C).

TABLE 2. *Estimates of percentage of aberrant anaphase I divisions in selected lines and in their backcrosses to parent species,* Nicotiana langsdorffii (P_1) *and* N. sanderae (P_2)

Type	Small	Intermediate	Large
P_1, F_1, P_2	3.2 ±1.8	28.5 ±1.6	16.26 ±1.2
LS, F_{10}	6.8 ±1.7	13.4 ±1.6	9.1 ±1.2***
langs. × F_8	27.2 ±1.6*	50.5 ±1.8	17.9 ±1.1
F_8 × *sand.*	5.1 ±1.0**	12.8 ±0.8	4.0 ±0.6
LS_1 × LS_2, F_7	10.2 ±1.4		
LS_1 × LS_3, F_7	1.2 ±0.4		
LS_2 × LS_3, F_7	3.9 ±0.6		

* LS_1, F_7 × *langs.*
** LS_1, F_7 × *sand.*
*** LS_2, F_9.

the F_1 hybrid, *N. langsdorffii* × *N. sanderae,* the *langsdorffii* backcrosses of the small and the intermediate selected lines show irregularities of the order of the F_1 hybrid and greater, respectively. The frequencies in corresponding backcrosses of the small and intermediate selection to *N. sanderae* are significantly less, as are the backcrosses of the large selection to either parent. A summary of the interrelationships based on cytological aberrations is presented diagrammatically in figure 4A.

The percentage of pollen abortion for both diploid and amphidiploid selected lines has been estimated in the F_8 and F_9 generations. Pollen samples of these selections consisted of shrunken and/or nonstaining grains, which were scored as nonviable, and well-filled, stained grains, which were considered functional. Mean percentages for selected lines as well as backcrosses are listed in table 3.

On the diploid level in the most advanced generation studied, the F_9, the three selected lines are each more sterile than *N. langsdorffii.* In relation to *N. sanderae,* only the small selection is significantly more fertile. Comparison between the F_8 and F_9 generations shows a significant change in fertility of both intermediate and large selected lines. In these instances, continued selection and inbreeding have been accompanied by increasing sterility.

In the F_9 generation on the amphidiploid level, all three selections exceed *N. langsdorffii* in pollen sterility, but form essentially one population with *N. sanderae.* The mean percentages of pollen abortion are remarkably similar in the F_9 generation, falling within the range 32 to 35 per cent. In contrast with the diploid selected lines, there is no evidence for sterility increasing with the number of inbred generations on the amphidiploid level. It should be noted that while each of the amphidiploids was more sterile than the corresponding diploid selected lines in the F_8 generation, only the small amphidiploid selected line is more sterile than the corresponding diploid in the F_9 generation. This changed relationship is due mostly to the increased sterility of the intermediate and large diploid selected lines.

The degree of sterility found in backcrosses to the two parental strains shows a relationship which is similar to that already described for aberration frequency. The percentage of aborted pollen tends to be higher in backcrosses to *N. langsdorffii* than in the corresponding *N. sanderae* backcross.

Backcrosses of the small and intermediate diploid selected lines with both parents

TABLE 3. *Estimates of percentage pollen abortion in selected lines and in their backcrosses to parent species,* Nicotiana langsdorffii (P_1) *and* N. sanderae (P_2)

Type	Small	Intermediate	Large
P_1, F_1, P_2	5.5 ±0.4	44.7 ±1.2	31.0 ±2.6
LS, F_8	24.0 ±1.4	23.0 ±2.3	29.4 ±2.2
F_9	20.3 ±2.2	50.0 ±6.5	69.9 ±3.6
langs. × F_5	53.5 ±1.8	55.7 ±4.9	—
langs. × F_7	42.1 ±1.5	45.6 ±2.3	49.2 ±1.4
F_5 × *sand.*	38.8 ±2.3	34.1 ±2.5	11.1 ±1.7
F_7 × *sand.*	40.4 ±2.7	30.2 ±1.9	12.8 ±3.1
LLSS, F_8	32.2 ±3.6	34.7 ±2.4	45.8 ±3.6
F_9	31.7 ±6.2	34.6 ±2.9	33.8 ±4.1
langs. × F_5	62.7 ±2.6	55.0 ±3.6	57.0 ±1.5
langs. × F_7	41.2 ±2.5	44.0 ±5.1	48.2 ±3.3*
F_5 × *sand.*	34.5 ±1.7	28.5 ±1.4	41.5 ±2.8
F_7 × *sand.*	51.3 ±3.2	36.1 ±2.3	34.8 ±3.3
LS_1 × LS_2, F_7	10.0 ±0.7		
LS_1 × LS_3, F_7	30.4 ±0.8		
LS_2 × LS_3, F_7	31.6 ±1.1		

* $LLSS_2$, F_8 × *langs.*

TABLE 4. *Estimates of crossability based on percentage of fruit set in pollinations among diploid selected lines (LS_1, LS_2, LS_3) and in their backcrosses to parent species,* N. langsdorffii *(LL) and* N. sanderae *(SS)*

Seed parent	Pollen parent				
	LS_1	LS_2	LS_3	LL	SS
Small selection, LS_1	60	100	0	0	100
Intermediate selection, LS_2	100	100	100	0	100
Large selection, LS_3	10	100	30	0	100
N. langsdorffii, LL	60	100	40	100	100
N. sanderae, SS	0	30	100	0	100

are partially sterile. The large corolla line, however, gives partially sterile progeny only in backcrosses to *N. langsdorffii* and should be considered essentially interfertile with *N. sanderae*. On the amphidiploid level, F_7 backcross progenies are all partially sterile, mean percentages ranging from 35 to 51 per cent. Interrelationships among the parental species and diploid selections, based on pollen abortion, are summarized diagrammatically in figure 4B.

CROSSABILITY

In addition to the above studies of meiotic irregularities and pollen abortion, crossability was chosen as a third means of estimating the degree of genetic isolation. Attempts were made at crossing the selected lines and parental lines in all possible combinations. Note was taken of the number of capsules produced and the data are expressed as percentage of fruit set in table 4.

Differences in crossability were found in that crosses between diploid selected lines and parental lines tended to produce fewer fruits than self-pollinations of the same selections. In those backcrosses where the pollen and seed parents differ significantly in length of style, no fruits were produced if the longer-styled type served as seed parent. It is known (Smith, 1937a) that there is a morphological isolating barrier which permits the short-styled *N. langsdorffii* to serve as seed parent, but not pollen parent, in the cross with the long-styled *N. sanderae*.

TABLE 5. *Estimates of means and standard deviation of the means for morphological characteristics of* N. langsdorffii, N. sanderae, *the F_1 hybrid, three diploid (LS) selected lines, and three amphidiploid (LLSS) selected lines*

	Character	*N. langsdorffii*, LL	*N. sanderae*, SS	F_1 hybrid, LS	Small selection, LS_1	Intermediate selection, LS_2	Large selection, LS_3	Small selection, $LLSS_1$	Intermediate selection, $LLSS_2$	Large selection, $LLSS_3$
Unselected	Height (cm.)	142.00±3.1	79.30±2.7	148.60±3.0	108.40±2.1	128.20±2.4	120.50±2.5	109.70±8.5	144.80±3.3	103.60±3.8
Vegetative	Stem diameter (mm.)	10.00±0.4	8.13±0.3	10.25±0.2	7.25±0.2	8.95±0.4	9.45±0.2	7.25±0.6	10.10±0.3	9.20±0.3
Vegetative	Branch number	47.30±2.9	10.90±1.2	37.90±1.4	30.80±1.4	28.80±1.3	9.90±0.5	14.60±1.6	8.70±0.3	18.70±1.7
Vegetative	Basal leaf length (cm.)	30.46±1.5	19.78±0.3	33.29±0.8	23.12±0.4	25.32±0.5	32.00±0.9	17.66±1.5	23.64±1.5	27.32±1.2
Vegetative	Basal leaf width (cm.)	13.38±0.6	9.02±0.2	15.82±0.5	12.50±0.4	12.84±0.4	13.15±0.5	9.09±0.8	12.15±0.7	13.38±0.9
Reproductive	Flower diameter (mm.)	14.60±0.2	66.40±1.3	30.90±0.7	15.20±0.4	20.40±0.3	49.45±1.0	22.15±0.6	33.50±0.9	49.45±1.1
Reproductive	Sepal length (mm.)	10.45±0.1	23.40±0.5	17.15±0.2	12.40±0.2	16.90±0.4	26.55±0.4	13.95±0.4	18.10±0.6	21.50±0.7
Reproductive	Limb length (mm.)	6.85±0.1	37.20±0.8	16.35±0.2	7.15±0.2	14.90±0.3	25.10±0.6	9.30±0.2	15.25±0.5	22.95±0.6
Reproductive	Stamen length (mm.)	22.50±0.1	81.05±1.5	40.45±0.3	26.50±0.2	36.65±0.5	84.75±1.1	26.85±0.6	42.10±1.0	51.15±1.7
Reproductive	Carpel length (mm.)	22.85±0.3	81.60±1.5	42.50±0.3	28.50±0.2	39.10±0.6	88.45±1.2	27.65±0.8	44.50±0.6	52.85±1.2
Selected	Tube base length (mm.)	5.10±0.1	60.40±1.2	19.50±0.3	8.80±0.4	20.45±0.4	63.40±1.0	10.40±0.7	20.65±0.9	30.95±1.3

The present observations indicate that a similar barrier is also present in the selected lines and serves to isolate the two parents from their derived selections. Crossability interrelationships are summarized diagrammatically in figure 4C.

Morphology

Morphological measurements were taken on ten unselected characters, consisting of five vegetative and five reproductive features, in the F_9 generation. Means based upon a single observation from each of ten plants are listed in table 5 with the appropriate standard deviation of the mean. Duncan's multiple range test (1955, 1957) was employed to test all possible differences among the sample means. The outcome of this procedure is shown in table 6, where means of these unselected characters and those of the selected character, tube length, are ranked in ascending order of magnitude. Any two means underscored by the same line in the table cannot be claimed to be significantly different, while any two means not underscored by the same line are significantly different.

This technique has served to separate the nine types into two or more significantly different groups. There are from one to four nonoverlapping groups among the vegetative characters, while for any one of the reproductive characters these lines have been separated into at least six such groups. The five unselected reproductive characters are more efficient, therefore, at differentiating the selected lines from their parents.

It can be observed that the ranking of means and the significant differences as shown in table 6 correspond closely for each of the reproductive traits. The dimensions of successive whorls of the flower, which are represented by length of sepals, limb, stamens, and carpel, appear highly correlated with the length of corolla tube base which has served as the criterion for selection.

Observations on morphological features have demonstrated that the six selected lines are well differentiated from the par-

TABLE 6. *Multiple range tests of ranked means for morphological characteristics of* N. langsdorffii (*LL*) N. sanderae (*SS*), *the* F_1 *hybrid* (*LS*), *three diploid selections* (LS_1, LS_2, LS_3) *and three amphidiploid selections* ($LLSS_1$, $LLSS_2$, $LLSS_3$)

Character	Ranking of means									Number of non-overlapping subsets
Height	SS	$LLSS_3$	LS_1	$LLSS_1$	LS_3	LS_2	LL	$LLSS_2$	LS	3
Stem diameter	LS_1	$LLSS_1$	SS	LS_2	$LLSS_3$	LS_3	LL	$LLSS_2$	LS	1
Branch number	$LLSS_2$	LS_3	SS	$LLSS_1$	$LLSS_3$	LS_2	LS_1	LS	LL	4
Basal leaf length	$LLSS_1$	SS	LS_1	$LLSS_2$	LS_2	$LLSS_3$	LL	LS_3	LS	3
Basal leaf width	SS	$LLSS_1$	$LLSS_2$	LS_1	LS_2	LS_3	$LLSS_3$	LL	LS	3
Flower diameter	LL	LS_1	LS_2	$LLSS_1$	LS	$LLSS_2$	$LLSS_3$	LS_3	SS	6
Sepal length	LL	LS_1	$LLSS_1$	LS_2	LS	$LLSS_2$	$LLSS_3$	SS	LS_3	7
Limb length	LL	LS_1	$LLSS_1$	LS_2	$LLSS_2$	LS	$LLSS_3$	LS_3	SS	6
Stamen length	LL	LS_1	$LLSS_1$	LS_2	LS	$LLSS_2$	$LLSS_3$	SS	LS_3	7
Carpel length	LL	$LLSS_1$	LS_1	LS_2	LS	$LLSS_2$	$LLSS_3$	SS	LS_3	7
Tube base length	LL	LS_1	$LLSS_1$	LS	LS_2	$LLSS_2$	$LLSS_3$	SS	LS_3	6

ental lines in a number of non-selected traits. It has also been found that these differences are more strongly expressed in the morphology of reproductive than vegetative organs. This may reflect, in part at least, the basis for selection which was solely the length of the corolla tube base.

Discussion

In the genus *Nicotiana,* two genetic devices which have been utilized during evolution in the proliferation of species are allopolyploidy and gene exchange (Goodspeed, 1954). Both processes require occasional interspecific hybridization. The evolution of this genus is characteristic of a phylogenetic line which consists of predominantly self-fertilizing species (Stebbins, 1957). In a previous paper, experiments were reported which explored the limits and consequences of allopolyploidy (Smith, Stevenson and Kehr, 1958). The present results deal with the consequences of hybridization between two predominantly self-fertilizing, low chromosome-number species which are sufficiently closely related to produce a partly fertile F_1, yet are diverse enough to give wide segregation in subsequent generations.

It was shown that through continued selection following a single interspecific hybridization an array of essentially pure breeding, diploid biotypes was produced which range widely in morphology in both selected and unselected characteristics. The variety of viable, fertile recombinants that could be produced from such a hybridization and subsequent multidirectional selection must clearly be very large. Such variability released by occasional interspecific hybridization among essentially homozygous self-fertilizing species in nature may endow a group of species with a breeding structure which is capable of striking a favorable evolutionary balance between fitness for present environments and flexibility for the future (Wright, 1931). Except in very uniform, stable environments, occasional recombinants would be expected to have an adaptive value sufficient for survival. In the absence of isolating mechanisms other than predominant self-fertilization, successful population structures may evolve (as in *Bromus carinatus, Senecio vulgaris,* and *Clarkia purpurea*) which are characterized by clusters of biotypes of subspecific rank (Stebbins, 1957). The breeding structure in the presently described experimental populations simulates a condition of rare hybridization, small effective population size, and strong selection pressure. This is conducive to rapid fixation of alleles.

It is evident from the results reported here that the partial reproductive isolation of the parental species has accompanied morphological divergence in the selected lines. The three criteria of reproductive isolation used, namely, crossability, meiotic chromosomal aberration, and pollen sterility, are not directly correlated. Certain general similarities can, however, be observed by comparing the three diagrams in figure 4, e.g., *N. langsdorffii* is the most strongly isolated by all three criteria.

Based on the ability of the three diploid selections to produce fruits in backcrosses, the data indicate that each selection is partially isolated from at least one parent, while the small selection is partially isolated from both parents. Based on the frequency of meiotic anaphase I aberrations, the small and intermediate selections are as well differentiated genetically from *N. langsdorffii* as *N. langsdorffii* and *N. sanderae* are from each other, but each is less well differentiated from the *N. sanderae* parent.

A single lagging chromosome was observed in backcrosses of the small and intermediate diploid selections to *N. langsdorffii.* This is presumed to be the *langsdorffii* D chromosome (Avery, 1938; Abraham, 1948; Lee, 1950). It is considered not to be present in the two selections and consequently is unpaired in the *langsdorffii* backcrosses. A high proportion of anaphase bridges, approxi-

mately one-third of aberrant cells, which occurred only in the backcross of the intermediate selected line to *N. sanderae* has been interpreted as evidence for an inversion heterozygote.

Backcrosses of each diploid selected line to *N. langsdorffii* yield between 40 and 50 per cent abortive pollen. On the basis of this criterion, they are as isolated as *N. sanderae* is from *N. langsdorffii.* The same is true of the backcross of the small diploid selected line to *N. sanderae.* The intermediate diploid selected line is less strongly isolated from *N. sanderae* and the large selected line shows no evidence of reproductive isolation from *N. sanderae* based on pollen abortion. The fact that certain diploid selections show a high percentage of pollen abortion in parental backcrosses, but relatively little chromosomal disturbance, is taken as evidence for the presence of genic, as well as chromosomal, sterility. For example, the small selected line in its backcross progeny with *N. sanderae* gave 5.1 per cent chromosomally aberrant cells as contrasted with 40.4 per cent abortive pollen grains.

What has been said of pollen sterility in backcrosses of the diploid selected lines is equally true in backcrosses of the amphidiploid selected lines. The only marked difference is that the backcross of the large amphidiploid selection to *N. sanderae* is more sterile than the corresponding cross on the diploid level.

One striking result of the inbreeding and selection program has been an increased pollen sterility in *N. sanderae* and in the intermediate and large diploid selections. That this is not a seasonal effect is clearly indicated by the absence of a similar trend in *N. langsdorffii,* the F_1, the small diploid selection, and all amphidiploid selections. An increase in sterility from 5–15 per cent to 31 per cent occurred upon inbreeding the strain of *N. sanderae* used, a self-compatible derivative from a stock originally segregating for self-incompatibility alleles (Smith, 1937a). This may reflect a gradual genetic imbalance caused by an increasing number of homozygous combinations in a genetic system adapted to a level of heterozygosity previously maintained by self-incompatibility. This result is similar to that observed by Bateman and Mather (1951) in inbreeding barley following a varietal cross.

A much higher level of pollen sterility (50 and 70 per cent) was reached in the intermediate and large diploid selections. This result has features in common with experiments showing increased sterility following selection in *Drosophila* for number of abdominal chaetae after crossing (Wigan and Mather, 1942; Mather and Harrison, 1949) and for number of sternopleural hairs following irradiation (Scossiroli, 1954). The correlated response of infertility with selection in the two larger flowered (more *sanderae*-like) types suggests either linkage or pleiotropy of genes for large corolla size which cause sterility when homozygous. The sample of biotypes is too small to verify that a preponderance of *sanderae* genes is crucial, but it is clear that some recombinant selections are markedly more infertile than others. The lack of any increase in sterility among the inbred amphidiploid selected lines, which remain variable to the F_9, may be confirmatory evidence of the relation between homozygosity and sterility.

The morphological data listed in tables 5 and 6 are of interest in showing the potentialities for character differentiation in unselected features under conditions of high variability, strong selection for one character, and a small effective population size ($N = 1$). Floral characteristics, all of which are probably related developmentally, show the same relative response. Characteristics of leaf length and width, which are morphogenetically related to floral features, show a loose correlation with the selected character, length of corolla tube base. Plant height and branch number are fixed in the selections at mean values which are unrelated to those of the selected character. They ap-

parently have been determined largely by random fixation.

A delayed response to selection is illustrated in figure 2 for the large selected line (LS_3). By the F_6 generation, corolla tube length in this line had not quite reached that of *N. sanderae* and the variance was no greater than in the homozygous parental species (Smith, 1954). No further response to selection was anticipated. Yet in the F_9 generation a large gain was realized and this was maintained in the F_{10} generation. The result is similar to that observed in selection experiments with *Drosophila* (Mather, 1942; Sismandis, 1942; Mather and Harrison, 1949). The most likely explanation is that polygenes, previously held together in a linkage block, are separated by crossing over in new positions along the chromosomes with resultant novel recombination occurring. As a consequence, new variability is released upon which selection can then act further to shift the mean.

The transgressive biotype (LS_3) and the introgressive transgressant developed by backcrossing it to *N. sanderae* are of interest as demonstrating potentialities of interspecific hybridization beyond that of producing intermediates. A genetic analysis of the transgressive biotype has been carried out by the junior author (Daly, 1958) and will be published elsewhere. Introgressive transgressants cannot be analyzed by methods based upon phenotypic intermediacy of hybrid derivatives (Anderson, 1949). The stabilization of polygenically controlled transgressive segregants, when combined with new, more simply inherited recombinations giving novel flower colors and patterns (Smith, 1937a), can result in populations which are markedly distinct. The specific parentage of such populations would be difficult to assess if encountered in nature. Since introgressive transgression is probably an important consequence following interspecific hybridization, the study of experimental populations is a valuable approach to evaluation of the evolutionary potentialities of hybridization. By this method, introgressive transgression can be investigated from its origin to ultimate genetic analysis.

Some examples of new morphological types of hybrid origin have been summarized by Stebbins (1950), Anderson and Stebbins (1954), and Clausen and Hiesey (1958). They include *Potentilla glandulosa, Ceanothus Jepsonii, Sitanion jubatum,* and species of *Viola, Iris,* and *Antirrhinum,* which are of known or hypothesized hybrid origin.

Summary

An analysis was made of diploid and amphidiploid populations of *Nicotiana* derived by interspecific hybridization, maintained by self-pollination, and selected on the basis of single plants having small, intermediate, and large corolla tubes. The parental species were *Nicotiana langsdorffii* and *N. sanderae*. The hybrid populations were advanced to the F_{10} generation.

On the basis of the selected character, the selected diploid lines were as uniform as the parental lines by the F_6 generation. Yet in the F_9 and F_{10} generation, the large diploid selected line showed significant advance and exceeded the size of the larger parent. The amphidiploid (segmental allopolyploid) selected lines remained highly variable throughout the experiment and showed relatively less response to selection.

Investigation of reproductive isolation based upon crossability, meiotic aberration frequency, and pollen abortion indicated that each selected line was isolated from its parents on the basis of at least one of these criteria. The most strongly isolated of the diploid selected lines, the small one, was as isolated from both parents as the parents were from each other on all criteria except one. Pollen abortion in backcrosses of the amphidiploid selected lines to the parental species was about the same as in backcrosses of the diploid selected lines.

Measurements were made on ten un-

selected characters in the F_9, five of which were characteristics of the flower and five were vegetative features. On the basis of the selected character, corolla tube base, the means were separable into six significantly different groups. This held essentially true for all floral characters, but there were fewer nonoverlapping groups among means of the vegetative characters.

The results demonstrate the nature of morphologically distinct and genetically isolated populations which can be derived by a single interspecific hybridization followed by strong selection in self-fertilizing populations.

Acknowledgments

The writers wish to acknowledge valuable aid received from Catharine P. Fussell and Harlan Q. Stevenson for cytological observations, from Douglas S. Robson for statistical analyses, and from Nancy G. Slack for collection and analysis of data.

Literature Cited

Abraham, A. 1948. A cytogenetical study of trisomic types in *Nicotiana langsdorffii*. Ph.D. thesis. Cornell University. 87 pp.

Anderson, E. 1949. Introgressive hybridization. New York, John Wiley. 109 pp.

——, and G. L. Stebbins, Jr. 1954. Hybridization as an evolutionary stimulus. Evolution, **8**: 378–388.

Avery, P. 1938. Cytogenetic evidence of *Nicotiana* phylesis in the *alata*-group. Univ. of Calif. Publ. Bot., **18**: 153–194.

Bateman, A. J., and K. Mather. 1951. The progress of inbreeding in barley. Heredity, **5**: 321–348.

Clausen, J., and W. M. Hiesey. 1958. Experimental studies on the nature of species. IV. Genetic structure of ecological races. Carnegie Inst. Wash. Publ. 615, Washington, D. C. 312 pp.

Daly, K. R. 1958. Genetic studies of biotypes derived from hybridization in *Nicotiana*. Ph.D. thesis. Cornell University. 193 pp.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics, **11**: 1–42.

——. 1957. Multiple range tests for correlated and heteroscedastic means. Biometrics, **13**: 164–176.

Goodspeed, T. H. 1954. The genus *Nicotiana;* origins, relationships and evolution of its species in the light of their distribution, morphology and cytogenetics. Chronica Botanica, **16**: 1–536.

Lee, R. E. 1950. A cytogenetic study of extra chromosomes in *Nicotiana Langsdorffii* and in crosses with *N. Sanderae*. Ph.D. thesis. Cornell University. 58 pp.

Mather, K. 1942. Polygenic inheritance and natural selection. Biol. Revs., **18**: 32–64.

Scossiroli, R. E. 1954. Effectiveness of artificial selection under irradiation of plateaued populations of *Drosophila melanogaster*. In I.U.B.S. Symp. on Genetics of Population Structure. Pp. 105–113.

Sismandis, A. 1942. Selection for an almost invariable character in *Drosophila*. Jour. Genet., **44**: 204–215.

Smith, H. H. 1937a. Inheritance of corolla color in the cross *Nicotiana Langsdorffii* by *N. Sanderae*. Genetics, **22**: 347–360.

——. 1937b. The relation between genes affecting size and color in certain species of *Nicotiana*. Genetics, **22**: 361–375.

——. 1943. Studies on induced heteroploids of *Nicotiana*. Amer. Jour. Bot., **30**: 121–130.

——. 1952. Relation between sterility and morphological characters in an interspecific *Nicotiana cross*. Genetics, **37**: 26–38.

——. 1954. Development of morphologically distinct and genetically isolated populations by interspecific hybridization and selection. Caryologia, 6 Suppl.: 867–870.

——, H. Q. Stevenson, and A. E. Kehr. 1958. Limits and consequences of multiple allopolyploidy in *Nicotiana*. Nucleus, **1**: 205–222.

Stebbins, G. L., Jr. 1950. Variation and Evolution in Plants. New York, Columbia Univ. Press. 643 pp.

——. 1957. Self fertilization and population variability in the higher plants. Amer. Nat., **91**: 337–354.

Wigan, L. G., and K. Mather. 1942. Correlated response to the selection of poylgenic characters. Ann. Eugen., **11**: 354–364.

Wright, S. 1931. Evolution in Mendelian populations. Genetics, **16**: 97–159.

AREA, CLIMATE, AND EVOLUTION

PHILIP J. DARLINGTON, JR.
Museum of Comparative Zoology, Harvard University

Received February 3, 1958

INTRODUCTION

This is an attempt to find what the geographical distribution of animals can tell about the evolution of dominant, successful animals.

Geographical distribution and evolution are very closely connected. Historically, animal distribution (the geographical patterns of it) provided some of the first, decisive evidence that convinced Darwin and Wallace of the fact of evolution (Darlington, 1959). In fact, evolution has made many of the patterns of animal distribution, and the patterns can still tell us new things about how evolution occurs. The particular question I shall ask and try to answer now is, where do dominant animals evolve: in large or small areas, and in warm or cold, stable or cyclical climates? The answer may tell not only where but also something about how dominant animals evolve.

Three kinds of evolution. Evolution can be divided (arbitrarily—it is an oversimplification) into three kinds (Darlington, 1948: 109; 1957: 565): speciation (which may or may not involve adaptation), adaptation to special environments, and general adaptation. General adaptation includes all the general improvements of structures and functions that increase animals' efficiency in many environments, that (for example) increase the speed of reactions or the efficiency of reproduction. It is adaptation to the general environment of the world, and it should lead to general dominance, to success over great areas and in many special environments.

Dominant animals are conspicuously successful ones. Dominant groups are usually numerous in individuals and numerous in species, often (eventually) diverse in adaptations, and often (eventually) widely and continuously distributed in diverse habitats. Also, of course, they are relatively successful in competition. The history of dispersal of animals seems to be primarily the history of successions of dominant groups, which in turn evolve, spread over the world, compete with and destroy and replace older groups, and then differentiate in different places until overrun and replaced by succeeding groups. (This is, of course, another oversimplification—radiation of successive dominant groups is only the main pattern among many patterns of animal dispersal.) The question (asked in different terms above) is, where do the dominant animals evolve in relation to area and climate?

Darwin and Matthew. Darwin was the first important evolutionary zoogeographer. A hundred years ago, in *The Origin of Species* (1859: in the two chapters on geographical distribution and in parts of other chapters; see also Darlington, 1959) he showed clearly how the geographical distribution of animals is related to evolution. The next really important treatment of the subject was by Matthew (1915) in *Climate and Evolution.* Matthew was a distinguished paleontologist. His work on fossil mammals was extensive and very good, and his ideas about the permanence of continents and the histories of islands were right and deservedly influential. In the following pages I shall have to pick out and criticize his theory of the climatic control of evolution. When I do it, readers should remember that this theory is a very minor part of Matthew's work. To judge Matthew just by his climatic theory (if it turns out to be wrong) would be as unfair as to judge Wordsworth by selections

from *The Stuffed Owl,* an anthology of bad verse by good poets. Repeatedly in the following pages I shall go back to Darwin, compare his ideas with Matthew's, and compare the ideas of both men with current ideas as set forth in my recent *Zoogeography* (1957).

Patterns of dispersal. I shall assume for present purposes (but only for present purposes—matters like this will never be beyond re-examination) that the continents and climatic zones have been constant *in position* during the period under consideration, which is mainly the later Tertiary, Pleistocene, and Recent, but that the north temperate zone was colder than now at times in the Pleistocene and warmer than now before that. Darwin, Matthew, and (probably) most modern zoogeographers agree about this.

Both Darwin and Matthew thought that animal (and plant) dispersal follows a main pattern, that successive dominant groups usually evolve (become dominant) in certain places and move (spread) in certain directions. Darwin drew his evidence primarily from the behavior of plants but extended his conclusions to organic "productions," by which he probably meant to include animals. He detected world-wide movements from north to south and from continents to islands, and his explanation was (1859, 1950: 322), "I suspect that this preponderant migration from north to south is due to the greater extent of land in the north, and to the northern forms having existed in their own homes in greater numbers, and having consequently been advanced through natural selection and competition to a higher stage of perfection or dominating power than the southern forms." In other words Darwin thought that *large area* induces evolution of dominant plants and animals.

Matthew drew his evidence primarily from the distribution and fossil record of vertebrates, chiefly mammals. He too detected a general movement from north to south, but he thought it was due to evolution of dominant groups north of the tropics, during the dry, cool phases of climatic cycles. In other words Matthew thought that *northern cyclical climate* induces evolution of dominant animals.

When I considered this matter recently (1957), it seemed to me that much evidence pointed to the main Old World tropics (tropical Asia and Africa) as the usual place of origin of dominant groups of vertebrates, including warm-blooded ones, and that the dominant groups spread from there in all directions that the land allows: to South Africa; across the islands to Australia; northward, to North America, and thence to South America; and from all the continents to any islands that could be reached. And my explanation was Darwin's, sharpened and extended: that dominant animals usually evolve in large, favorable areas and move (spread) to smaller and/or less favorable areas. In other words I thought that *large area and favorable climate* induce evolution of dominant animals. By "favorable" I meant physically favorable to existence of life. I suppose the conditions of existence are most favorable in the tropics, less so in colder or less stable climates. It does not necessarily follow that dominant animals evolve in the physically most favorable climates, but I think that in fact they do. Matthew thought not. He thought (1915, 1939: 7) that dominant groups should evolve under "conditions . . . unfavorable to abundance of life and the ease with which animals could obtain a living."

Matthew's suggested pattern of dispersal (A) and mine (B) are compared in figure 1. If they were meaningless shapes, these patterns would not seem very different, but they are not meaningless. They imply different things about evolution. Pattern A implies that dominant animals evolve in response to northern cyclical climates; B, that they evolve in large areas and favorable, relatively stable climates. These two patterns would not be reconciled if tropical or subtropical climate extended into the north-temperate zone, as it formerly did. Matthew's thesis

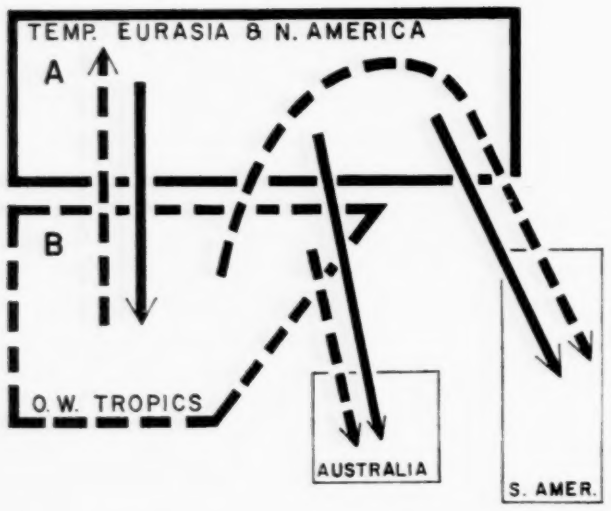


FIG. 1. Two suggested dispersal patterns: A (solid lines), with center in the north temperate zone (Matthew's pattern); B (broken lines), with center in the Old World tropics.

is that dominant vertebrates evolve in a zone above the tropics, when it is not tropical, and because it is not tropical.

Effect of area. Area—the relative sizes of different land masses—does affect the evolution and dispersal of dominant vertebrates, whatever the effect of climate may be. Vertebrates have moved back and forth over the world in very complex ways, but the sum of the movements has been from larger to smaller land masses. Evidence of this comes from many sources (Darlington, 1957: 545–547). For example, existing distributions suggest more movement of vertebrates from Asia to Australia than the reverse, more from the Old World to North America than the reverse, and more from North to South America than the reverse. Movements of vertebrates from continents to islands have been even more strongly directional: existing distributions show that many vertebrates from all the continents have reached islands across moderate water gaps, while very few have returned from islands to continents. In some cases the fossil record shows general (average) directions of movement, for example movement of mammals from Asia to North America during much of the Tertiary (Simpson, 1947) and from North to South America since the late Pliocene (Simpson, 1940; and see below, p. 491). All this concerns natural dispersal, in the past.

There is additional evidence in the behavior of plants and animals dispersed by man. Darwin (1859, 1950: 286) says, "From the extraordinary manner in which European productions have recently spread over New Zealand, and have seized on places which must have been previously occupied, we may believe, if all the animals and plants of Great Britain were set free in New Zealand, that in the course of time a multitude of British forms would become thoroughly naturalized there and would exterminate many of the natives. On the other hand, from what we see now occurring in New Zealand, and from hardly a single inhabitant of the southern hemisphere having become wild in any part of Europe, we may doubt, if all the productions of New Zealand were set free in Great Britain, whether any considerable number would be enabled to seize on places now occupied by our native plants and animals. Under this point of view, the productions of Great Britain [i.e. of Eurasia] may be said to be higher [more dominant] than those of New Zealand. Yet the most skillful naturalist from an examination of the species of the two countries could not have foreseen this result."

This is a sample of a kind of evidence that tells much about the relative dominances of organisms of different parts of the world. Man, intentionally or by accident, has carried many plants and animals, especially weeds and insects but also some vertebrates including some mammals and birds, all over the world, back and forth in every direction. But man-carried species have taken hold in some directions more than others. Species from Eurasia have probably established themselves in North America more than the reverse (I am not quite sure about this). Species from Eurasia and North America have certainly established themselves in Australia and probably also in southern South America more than the reverse. And species from continents have estab-

lished themselves on islands more than the reverse. This pattern follows area. The plants and animals of larger areas have usually established themselves in smaller areas, although there have been some reverse cases too. This—the behavior of living plants and animals—is good evidence that dominant forms usually evolve in large areas, although the matter is much more complicated than my summary of it and needs detailed study.

I have been asked whether movements of animals between different parts of the world are simply proportional to the areas or the faunas concerned. They are not.

Take, as a hypothetical example, two areas, one ten times the size of the other (10A and 1A), and suppose they have faunas limited by area to 2N and 1N species respectively (see p. 504 for reasons for selecting these figures), and suppose no extinction occurs in either fauna. Then there can be no exchange. The larger fauna has more to give, but the smaller fauna has as many species as its area allows and no more can be added.

Now suppose that extinction (but not evolution) occurs. If each fauna consists of dominant species (D, capable of spreading) and undominant ones (E, liable to extinction) in the same proportion, so that the faunas are $2(D + E)$ and $1(D + E)$ respectively, one unequal exchange can occur in which twice as many species (D's) spread from the large to the small fauna as the reverse, the additions to each fauna being balanced by extinction of E's (if there are enough E's in the smaller fauna). Then no further exchange can occur.

Now suppose that evolution as well as extinction occurs, more effectively in the 2N than in the 1N fauna. Then exchange will become unequal again, but the ratio of exchange will not be fixed. It will depend, partly, on time. If the two faunas are separated for a short time and then allowed to exchange, the exchange may be nearly equal. But if they are separated for a long time, during which evolution steadily increases the relative dominance of the larger fauna, the eventual exchange may be very unequal or may even approach a one-way movement.

A very unequal exchange of mammals has actually occurred between North and South America, after the South American fauna had been isolated for sixty million years or more (Simpson, 1940). When the two continents became connected, perhaps a couple of million years ago, many North American mammals moved (extended) to South America. Some South American ones moved northward too, but they were relatively few and most of them later became extinct in North America. The net result is that, while recent invaders from North America now make up about half the mammal fauna of South America (Darlington, 1957: 333, table 7), only three species of South American mammals now exist in North America (excepting bats and a few terrestrial forms that reach the tropical southern edge of North America). Moreover there has been extensive replacement in South America. All the old South American (marsupial) carnivores have been replaced by invading carnivores. All the old South American hoofed mammals have been replaced by invading ungulates. And some other South American mammals have probably been replaced by invaders. But of the three South American species now in North America, two (the armadillo and porcupine) have specialized food habits and may have moved into unoccupied niches without replacing anything else. Only the oppossum is an unspecialized, presumably competitive invader!

This exchange has really been between the enormous area of Megagea, which includes North America, and the much smaller area of South America. Many of the groups of mammals that have invaded South America came originally (through North America) from the Old World. But no South American mammal has returned to the Old World. As between the Old World and South America,

the exchange has, in fact, been a one-way movement.

Darwin suspected that large area induces evolution of dominant plants and animals (above, p. 489). Matthew apparently did not suspect it. Matthew's pattern of dispersal (fig. 1, A) runs mostly from large to small areas, but he was apparently unaware of Darwin's explanation, and it apparently did not occur to Matthew that area as well as climate might be involved in evolution of dominance. Matthew (1915, 1939: 140) does mention "wider area" as favoring "expansive evolution" (radiation or diversification of dominant groups), but not as favoring evolution of dominance. He thought that dominant animals were made by northern climatic cycles, and that they then diversified in and spread from large central areas in the north. Nevertheless, having noted Matthew's apparent lack of agreement (I think he did not so much disagree as fail to consider the possibility), I am going to assume as a working hypothesis justified by the evidence that area is an important factor (but not the only one) in evolution of dominance, and that, other things being equal, the most dominant animals usually evolve in the largest areas. This leaves climate to be considered.

Effect of climate. We now need a biologists' definition of "the tropics." Many definitions are possible and no simple one is entirely satisfactory, for the tropics vary in climate (wet to dry), vegetation (rain forest to desert), and animal life. The diversity of the tropics is well described by Aubert de la Rüe *et al.* (1957). However we do not need a full or precise definition, but one that will emphasize the significant differences between the tropics and the north temperate zone. For this purpose the tropics may be defined as the zone in which, compared to the temperate zone, temperatures are relatively uniformly warm by day and night and at all seasons (and have probably been relatively uniform for a long time in the past), and in which plants and animals are relatively numerous, diverse, and diversely adapted, reaching a maximum in number of species, diversity, and interadaptation in the rain forest—the tropics could be defined as the zone within which, when other conditions are suitable, warmth and stability of temperature permit development of what we call tropical rain forest with its associated complex fauna. This zone now lies mainly between the Tropics of Cancer and Capricorn, but, biologically, its boundaries are irregular and in part poorly defined, and the tropical zone was certainly wider than now and perhaps less well defined at times in the past. But exact boundaries are not important for present purposes. Both temperature and plant and animal life are irregularly graded from the equator to the arctic (fig. 2). At the tropical end of the gradient temperature is relatively warm and stable, and floras and faunas are large and complex; at the northern

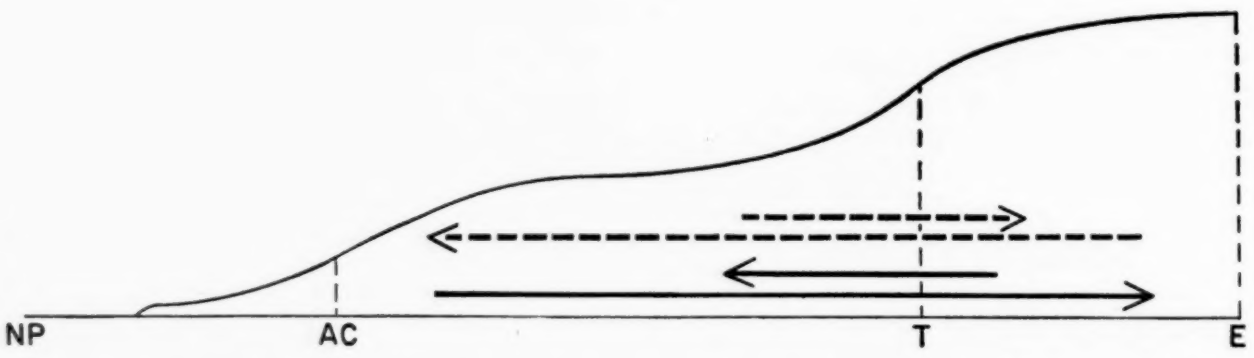


FIG. 2. Gradient of number of species in relation to climate, based (roughly) on number of mammals in eastern Asia. NP, North Pole; AC, Arctic Circle; T, Tropic of Cancer; E, Equator. Solid arrows suggest expected movements if dominant groups evolve mostly in the north temperate zone; broken arrows, if they evolve mostly in the tropics.

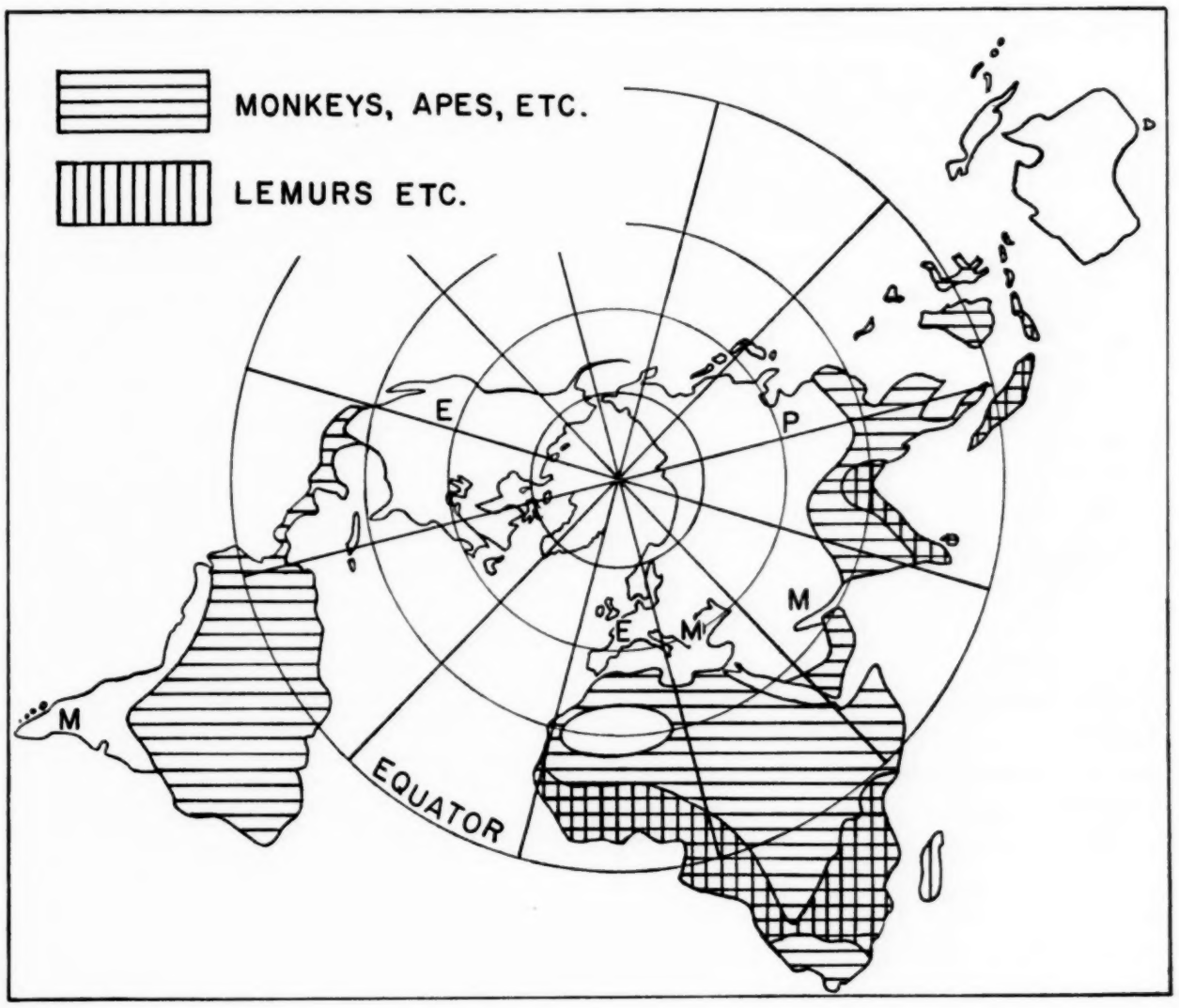


FIG. 3. Distribution of primates other than man, after Matthew (1939:46), redrawn, slightly simplified. Letters show approximate localities of northernmost and southernmost fossil primates in: E, Eocene; M, Miocene; P, Pliocene.

end, temperature is colder and less stable (fluctuating daily, seasonally, and during recent geological time), and floras and faunas are relatively small and simple. The question is not the position of dividing lines but at which end of the gradient dominant animals usually evolve and in which direction they usually disperse. Stated differently, the question is whether evolution of dominant animals occurs more in simple faunas and unfavorable, unstable climates (as Matthew thought) or in complex faunas and favorable, relatively stable climates. ["Favorable" here refers to conditions of existence, not to factors affecting evolution.]

The gradient (fig. 2) is probably steeper now than it has usually been in the past, but a less steep gradient, reflecting a fundamental relation between climate and the size and complexity of floras and faunas, has presumably always existed. Climate has apparently tended to restrict exchange of mammals between Eurasia and North America at least since the later Eocene (Simpson, 1947: 220), which suggests that the gradient has been steep enough to have a marked effect on faunas at least since then. This is as far back as we need to go for present purposes.

Climate can limit animal distributions without involving evolution. Figure 3 is Matthew's map of the distribution of primates, other than man. Matthew considered this distribution radial from an Holarctic center, with primitive forms (lemurs etc.) in a "marginal position." But figure 4 is the distribution of the same primates on an orthographic pro-

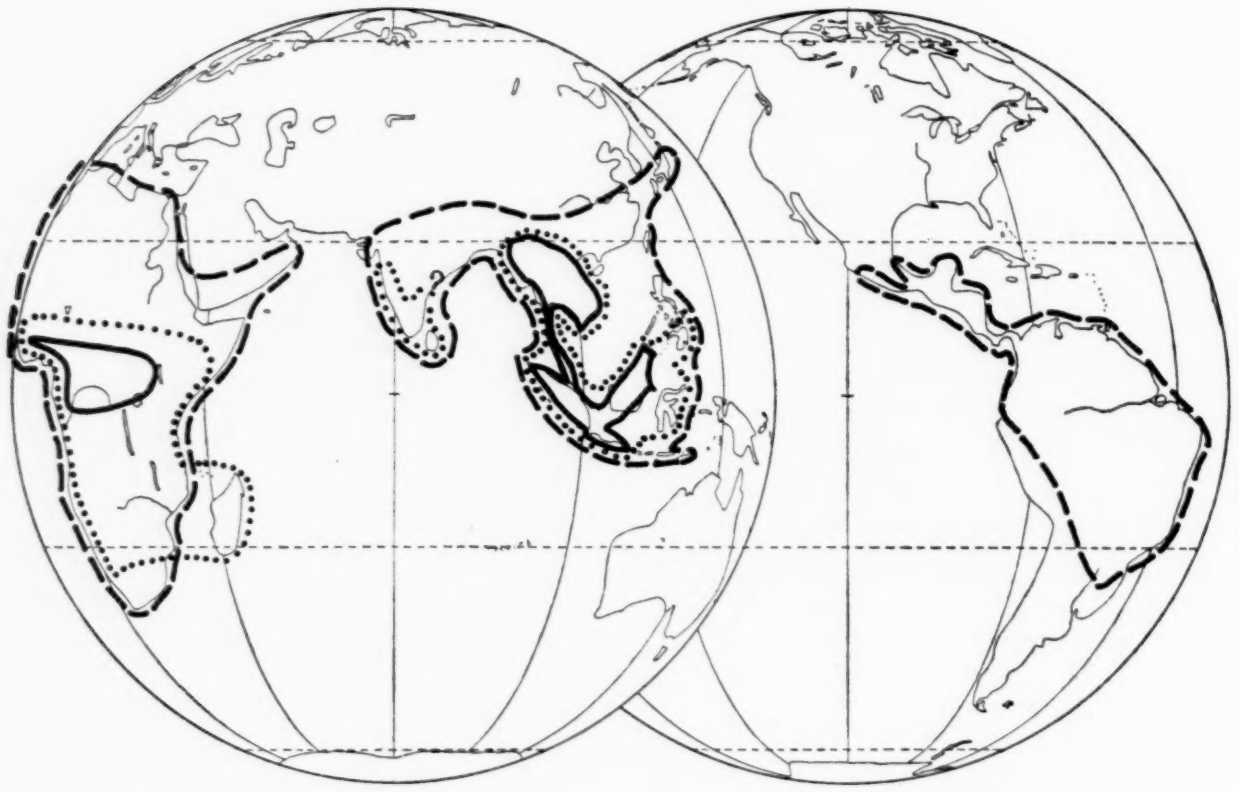


FIG. 4. Distribution of primates other than man, on an orthographic projection, with details brought up to date. Dotted lines are approximate limits of existing Prosimii (lemurs, tarsiers, etc.) ; broken lines, of monkeys etc.; solid lines, of anthropoid apes.

jection, with details brought up-to-date. On this map, it looks as if the effect of climate is primarily to limit distribution both northward and (in South America) southward. The lemurs etc. (Prosimii) in Africa and the Orient no longer seem marginal; they are within the limits of distribution of higher primates; they inhabit all the big, accessible forests of the Old World tropics; on the continents, in fact, existing Prosimii are no more marginal that the great apes, which Matthew thought represented the last prehuman cycle of dispersal from the north. The lemurs on Madagascar are marginal, outside the geographical limits of other primates, but this is presumably an effect of geographical isolation, not of dispersal with regard to climate. Fossils show that primates extended both farther north and farther south than now at times in the Tertiary, but that need mean only that limits were wider when climates were warmer. The record is too limited and too one-sided geographically to show whether or not particular primates were in the Old World tropics at critical times in the Tertiary. Climate plainly sets general limits to primate distribution and probably affects many details of it. But does the distribution of primates (fig. 4) really show a pattern of successive radiations from the north, first of lemurs (dotted lines), then of monkeys (broken lines), and then of great apes (solid lines)? I think a fair answer is that there certainly have been successive radiations of primates, that the present distribution of primates does not forbid northern origins, but that their distribution does not form a clear pattern of successive radiations from the north and therefore is not evidence that northern climatic cycles have controlled the evolution and dispersal of primates. This example suggests that Matthew did not sufficiently distinguish the present limiting effect of climate from its supposed effect on evolution in the past. What we want now is evidence of the effect of climate *on evolution*.

The Evidence

Where is the evidence? If area is a factor, the effect of climate on evolution should be looked for in places where area has comparatively little effect. Movements from the great continents to Australia or South America are probably influenced by relative areas and are not good evidence of effect of climate: patterns A and B of figure 1 agree that movement is from the great continents to Australia and South America. It is within the limits of the great continents, where A and B are different, that we should look for evidence of the effect of climate on evolution. What we want is evidence of whether dominant groups usually evolve in the north temperate zone or in the Old World tropics. And we want especially the evidence of warm-blooded mammals and birds, because they are the ones there is most doubt about, and because Matthew based his theory mainly on the mammals.

Evidence of the fossil record. The best evidence might be expected to come from fossils. Is the fossil record good enough in the north temperate zone and the Old World tropics to show directly in which place dominant groups have usually evolved or in which direction (north or south) they have usually moved? Mammals have the best fossil record of any animals. In parts of the north temperate zone it is very good, but in the Old World tropics the record even of mammals is very poor. Matthew knew this. He says (1915, 1939: 19), "In the Oriental region, we know nothing of the land life of the early Tertiary (except for a glimpse at the Eocene fauna of Burma), and in the later Tertiary we know only the life of its northern borders, . . ." and "In the Ethiopian region, we have but a single glimpse of the (early) Tertiary land fauna, and that is derived from Egypt, ..." We know a little more now than Matthew did, at least in Africa (Hopwood, 1954; Leakey *et al.*, 1955), but it is still true that in the whole of the Old World tropics there is no record of mammals in the very early Tertiary and only a fragmentary one later. Consider what this means geographically. Suppose (this example is imaginary) that there were ten times as many mammals known fossil in the north temperate zone as in the Old World tropics and that everything else were equal, then, with incomplete samples and on the basis of chance alone, we should expect to find about ten groups known first in the north to one known first in the tropics. This would have nothing to do with place of origin or direction of movement, but it might be misinterpreted! The evidence of fossil mammals, then, is one-sided, very scanty in the tropics, and very difficult to interpret. The fossil record of birds is practically useless for present purposes.

Evidence of selected groups. Most of Matthew's evidence was presented as a series of histories of separate groups of mammals and other vertebrates. Each history was derived from the present distribution and fossil record of the group concerned. This should be the perfect method, if there is enough evidence, and if it is used properly. One of Matthew's histories, that of the primates other than man, has been discussed and criticized above (p. 493). In this case the method did not seem to work well. The distribution of primates looked radial on Matthew's map (fig. 3) but not (or not so much so) on a more world-like projection (fig. 4), and, although climate seemed to set limits to primate distribution, the distribution did not seem to form a clear pattern of successive waves of dispersal from the north. Moreover the fossil record of primates seemed too limited and too one-sided geographically to show places of origin and directions of movement within the main part of the Old World. The distribution of primates might be reconciled with a history of successive dispersals from the north but does not seem to give real evidence of it. This,

I think, is the best that can be said of most of Matthew's histories. They might be reconciled with radiation from the north but most of them seem to give no real evidence of it. Most of the histories (like that of the primates) are weakened by failure to distinguish the present limiting effect of climate from its supposed past effect on evolution and by failure to distinguish the effects of relative area and of geographical isolation from the effect of climate, as well as by failure to allow for the great gaps in the fossil record in the Old World tropics.

It is unnecessary to criticize all Matthew's other cases in detail, but I shall review the apparent histories of a few selected groups: man, elephants etc., cattle etc., horses, and murid rodents. Elephants and horses have been selected because they have exceptionally good fossil records. Man, cattle, and murids have been selected because they are probably the most recently dominant groups of mammals, most likely to give clear evidence of where and how dominant groups evolve. In studying the evolution of dominant animals we should, of course, give most attention to the most dominant groups.

Man. At first thought it seems as if man has recently risen to predominance in the north temperate zone. North temperate man now can atomize anyone else. But this is not what is meant here by dominance. Dominance is a biological matter, and the test of it is in the evolution and movements of populations. There are two pertinent questions to ask. Has the main course of man's evolution, the long rise to dominance during the last million years, been primarily in the Old World tropics or north of them? This question cannot be given a final answer but the evidence seems to favor the tropics. The distribution of a few human bones and many stone tools shows that man has been wide-spread in the Old World tropics probably throughout his history. We do not know how far northward he extended until recently, but apparently neither he nor his immediate ancestors got far enough north to reach America until almost the end of the Pleistocene. The other question is, are human populations now moving from north temperate regions into the Old World tropics, or vice versa? I do not know the answer; certainly the owners of atomic and fusion bombs are not moving into the tropics of Africa and Asia in very large numbers. (Matthew thought that "the center of dispersal of the human race" was in Asia probably north of the Himalayas, but he oversimplified human history and did not know what is now known of the occurrence of man in Africa as well as tropical Asia in the Pleistocene.)

Elephants etc. Proboscideans—elephants, mastodonts, mammoths, etc.—first appear fossil in Egypt, in the Upper Eocene and Lower Oligocene, but had probably been evolving and diversifying in Africa for some time before that. Their history since then has been one of spectacular radiation from Africa or at least from the main part of the Old World. As new groups arose, they spread rapidly, and most of them sooner or later (from the Miocene to the Pleistocene) extended to North America, although a few groups did not. Probably at least a dozen groups reached North America, and eventually (in the late Pliocene and Pleistocene) three or four reached South America too. In the Pleistocene proboscideans were still numerous on all continents except Australia and in cold as well as hot climates, but in the short time since then they have been reduced to the two existing elephants, in different genera, in Africa and tropical Asia. (Matthew, without much evidence, questioned the African origin of proboscideans and thought they might have originated in southern Asia and later transferred their dispersal center northward, a "quite exceptional" history that did not fit his pattern.)

The geographical history of one group of proboscideans, the mastodonts, has been diagrammed by Simpson (1940: 143) (my fig. 5). Many details of this diagram

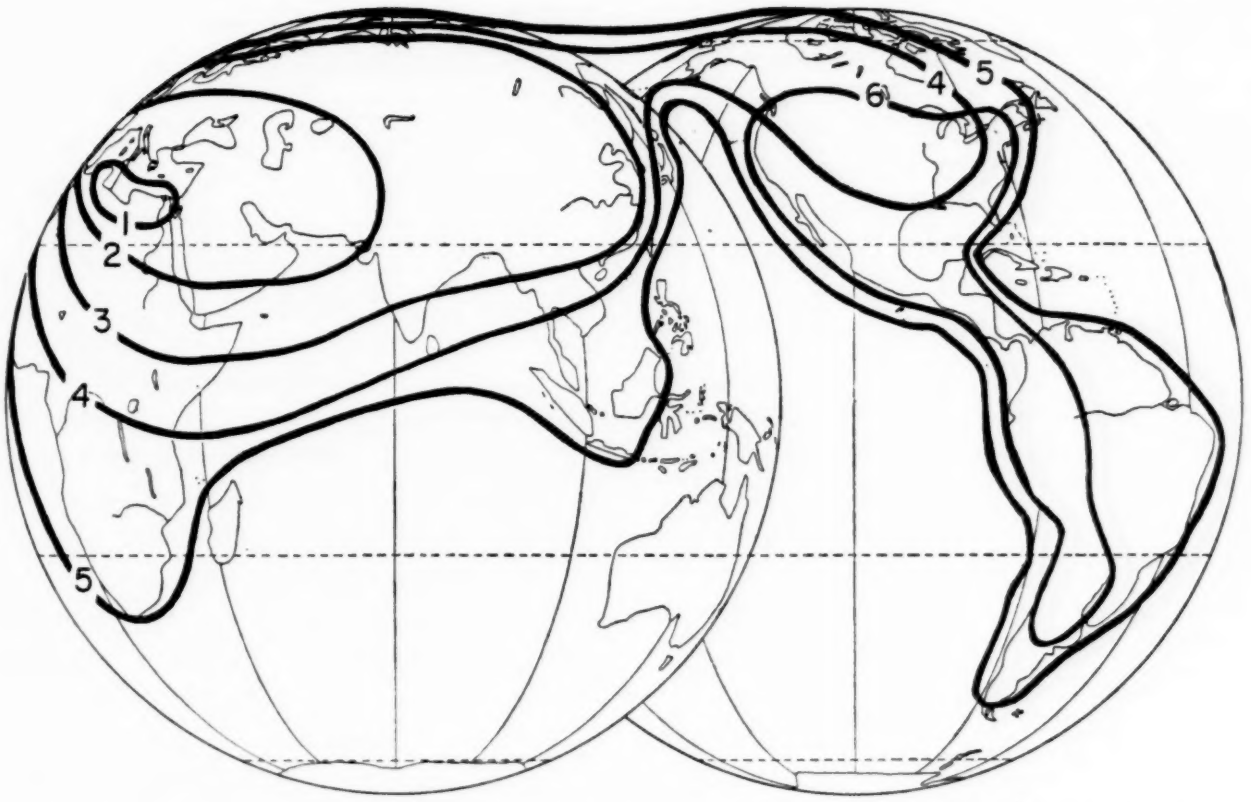


FIG. 5. Geographical history of mastodonts, diagrammatic, after Simpson (1940: 143), slightly simplified and transferred to an orthographic projection. Distributions are suggested in: 1, Oligocene; 2, earliest Miocene; 3, later Miocene; 4, Mio-Pliocene; 5, Plio-Pleistocene; 6, late Pleistocene. Many details are hypothetical (see text).

are necessarily doubtful. For example it is doubtful if mastodonts were ever confined to a small northern corner of Africa. It seems more likely that they arose in the main part of Africa, where there is no pertinent fossil record. Nevertheless the diagram does suggest the probable history of one group of elephant-like mammals. And it illustrates a kind of geographical history that may have occurred in other cases. In this case the mastodonts apparently began in Africa, then spread through the warmer part of Eurasia, and then spread far enough north to reach America. Other groups of animals may have followed the same main pattern, in some cases beginning in tropical Asia rather than in Africa, or they may have begun in the same way but never spread far enough north to reach America. Failure of a dominant Old World group to reach America suggests that it has evolved primarily in the warmer part of the Old World.

Cattle etc. The family Bovidae (cattle, antelopes, sheep, goats, etc.) is the most dominant existing family of hoofed mammals and one of the most recent of all mammal families to evolve. Bovids are now most numerous and diverse in Africa, less so in Eurasia (tropical and temperate), and very few in North America. Their fossil record begins in the Miocene and is extensive, but is probably very incomplete in Africa. Several tribes that are fossil in Europe and Asia are now confined to Africa, but this is evidence of withdrawals rather than of place of evolution. Simpson (1945: 157–162) lists 134 fossil or living genera of the family in the Old World. Only five of them have reached North America, where one additional genus is endemic. The present distribution (concentrated in Africa) and the fact that so few stocks have reached America (apparently none before the Pleistocene) suggest that the family has evolved, very complexly, mainly in the warmer part of the Old World. (Matthew thought that the centers of dispersal of antelopes etc. and of cattle have been in southwestern and southeastern Asia re-

spectively, but he oversimplified bovid history and did not allow for the poverty of the fossil record in the tropics.)

Horses etc. Equids (horses, zebras, asses, and their ancestors) appear first in the Lower Eocene of Eurasia and North America. It is probable (but not absolutely certain) that the whole main line of further evolution of equids was in temperate North America. Their evolution was moderately complex (but much less so than that of some other families of mammals), with several successive radiations of genera. Several genera spread from North America to Eurasia, and at least one reached Africa in the Pliocene, but all the older genera that reached the Old World became extinct. Then most of the five or more Pliocene stocks became extinct even in North America; but one survived, radiated again, and produced *Equus* in North America (still in the Pliocene); and *Equus,* with numerous species, spread widely over the world, extending through Eurasia to Africa. (*Equus* and one or more other equids reached South America too in the late Pliocene or Pleistocene.) Finally, all equids became extinct in North (and South) America, leaving only the few existing species of *Equus* in temperate Eurasia and in Africa. This well documented history clearly follows Matthew's pattern of evolution in the north, although the invasions of the Old World tropics have not been extensive.

Murid rodents. Within their ecological limits, rodents are dominant mammals. The family Muridae (common Old World rats and mice) is probably the most recent family of rodents to become dominant. It includes the Black and Norway Rats and the House Mouse, which have spread over the world with man. However the fossil record of the family is so poor that its history has to be deduced almost entirely from its present distribution. Murids are numerous and diverse throughout the Old World tropics. Several different ones have reached Australia at different times since (perhaps) the late Miocene, which suggests that the family has been dominant in tropical Asia since then. Murids occur also in temperate Eurasia but, in spite of their dominance, they have not reached America except as carried by man, which suggests that they have never been numerous in the north and that the family has evolved primarily in the warmer part of the Old World. (Matthew thought that "the myomorph families are evidently of Holarctic origin" but did not otherwise discuss the place of origin of the murids. He argued that the related cricetids are of northern origin but he did not consider the possibility that they may once have been world-wide and may have been replaced in the Old World tropics by murids.)

Summary of selected groups. The examples just discussed form two categories. The first includes dominant groups that seem to have evolved primarily in the Old World tropics or at least in the warmer part of the Old World: man, other primates (p. 493), proboscideans, bovids, and murid rodents. Of these groups only the proboscideans and perhaps the bovids have anything like an adequate fossil record. The other groups are placed here because their present distributions center on or include the Old World tropics and because they have not reached North America or have done so only infrequently or recently. Some other dominant existing mammals could be placed here for the same reasons: e.g. viverrids (civets, mongooses, etc.), pigs, and fruit bats.

The second category includes groups that have evolved in the north temperate zone or that seem to be more northern than tropical in evolution. Of the groups discussed, only the horses belong here. They have clearly evolved in the north and invaded the Old World tropics, but their invasions have not been extensive. Other families that might be placed here include camels and pronghorns, but they are primarily North American rather than north temperate and they are not known to have entered the Old World tropics at all. Bears and deer might be placed here

because they are well represented in the north and absent in Africa, but we do not know how they have evolved and moved within Eurasia. Some additional smaller or more specialized families (moles, pikas, beavers, jumping mice, etc.) might be placed here, but they are hardly to be counted as dominant groups.

A third category might be made for widely distributed dominant or formerly dominant groups in which there is even less evidence of geographical origins, whether in the Old World tropics, the north temperate zone, or both. This category might include shrews, weasels, cats, dogs etc., rhinoceroses, rabbits, squirrels, cricetid rodents, and some insectivorous bats, as well as many non-dominant and less well known groups.

Although this summary does not include all mammals and is to some extent subjective, it does include the best known and most recently dominant families, which should give the best evidence of where and how dominant groups evolve. What general conclusions can fairly be drawn from this evidence?

If my presentation and interpretation of this evidence is even approximately correct, the groups that have evolved in the Old World tropics and spread northward are, if not more numerous, certainly larger and more dominant than those that have evolved in the north temperate zone. However some, usually smaller groups do seem to have evolved in the north and some of them, at least the horses and perhaps a few others, have or may have spread into the Old World tropics. This suggests an unequal exchange, with most dominant groups rising in the Old World tropics and spreading northward but with some countermovement too. This is what the evidence seems to show, and an exchange is also what would be expected. The great faunal movements that have been analyzed are exchanges rather than simple directional movements. For example, Simpson's (1947) analysis of movement of mammals across Bering Strait during the Tertiary shows a series of complex exchanges, each apparently with more movement from Asia to America than the reverse, but each with some countermovement too; and Simpson's (1940) analysis of recent movements of mammals between North and South America again shows a complex exchange, with (finally) most movement from north to south but with a little countermovement.

But suppose my presentation of the evidence of selected groups is not convincing. Most of the separate cases are less than conclusive, and readers may feel that all of them together do not justify my general conclusion. Can these cases then prove Matthew's thesis, that most dominant groups of mammals have evolved in the north and spread into the tropics? I think not. Some of the cases might be reconciled with a history of successive dispersals from the north, but most of them seem to give no clear evidence of it, and certainly not proof (p. 496 above). This amounts to saying that, if the cases do not prove that tropical origins are the rule, they do not prove that northern origins are the rule either. This is a compromise (which, I think, does less than justice to the evidence of tropical origins) but it is also an important conclusion, and readers should consider very carefully whether or not they agree with it. It is important because most of Matthew's evidence, which he thought proved that dominant animals evolve north of the tropics, came from the histories of separate groups of mammals. If this evidence does not prove what Matthew thought it proved, that leaves little to support his thesis of northern origins.

We have now to find what other evidence there is and what it seems to show.

Evidence of whole faunas. The distribution of dominant families in whole faunas (of mammals and birds) in the north temperate zone and the Old World tropics might give evidence of place of evolution of dominant groups. If dominant groups usually evolve in the north, replacing older groups there while the older groups survive in the tropics (as

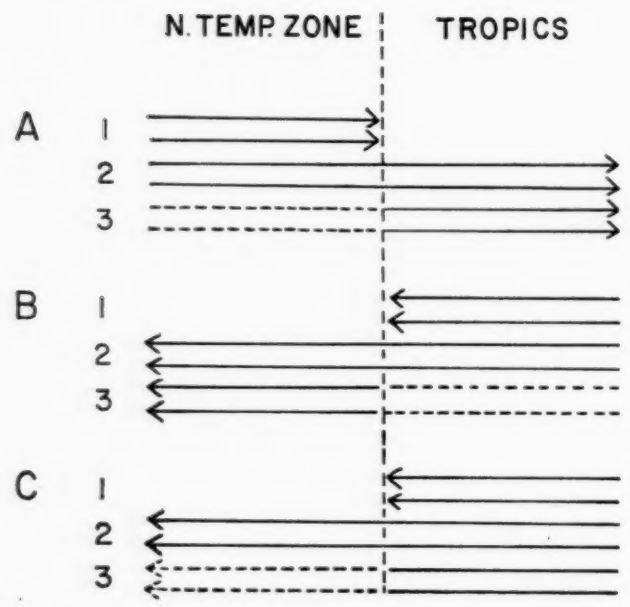


FIG. 6. Expected distributions of dominant families of mammals and birds in the north temperate zone and the Old World tropics: A, if successive dominant families evolve in the north and spread into the tropics, with replacements beginning in the north (Matthew's hypothetical pattern); B, if successive dominant families evolve in the tropics and spread northward, with replacements beginning in the tropics; and, C, if successive dominant families evolve in the tropics and spread northward, with replacements beginning in the north (the apparent actual pattern). Solid lines indicate present occurrence; broken lines, past occurrence. The numbers 1, 2, 3 indicate relative time, 1 being the first and 3 the last stage in the cycle of rise, spread, and beginning of replacement of dominant families.

Matthew thought), they should make a pattern of distribution something like figure 6A. But if the dominant groups usually evolve in the tropics, replacing older groups there while the older groups survive in the north, the pattern should be something like 6B. Arrowheads show the direction of movement in each case. If, as I have suggested, there is an unequal exchange rather than movement in a single direction, the patterns would be more complicated. We should therefore be prepared to find that no simple diagram fits the facts exactly, and if none does, we shall have to try to decide what diagram comes closest to fitting.

The existing mammal faunas of tropical Asia and of an approximately equal area of temperate eastern Asia differ in two ways (Darlington, 1957: 327–329). The tropical fauna is larger, containing nearly twice as many species as the temperate fauna; and the tropical fauna is more diverse, consisting of widely distributed families plus many additional families, while the temperate fauna consists of the same widely distributed families plus few additional ones. This is the general pattern of zonation of both mammals and birds: tropical faunas are large and diverse; temperate faunas are like tropical faunas from which much has been subtracted; and arctic faunas are like temperate faunas from which much more has been substracted (*op. cit.*: 341). The northern faunas have few important groups confined to them, except of water birds. This pattern is diagrammed in figure 6C. It is not 6A and not exactly 6B, but seems to be a modification of the latter. It would be formed if dominant mammals and birds usually evolve in and spread from the tropics, but if older groups usually begin their disappearance in the north, shrinking back into the tropics instead of forming a pattern (like 6B) of successive northward dispersals. This seems to be what does happen, among mammals and birds (*op. cit.*: 560–561). The proboscideans (above, p. 496) are an example.

Figure 6C is a simple model of a very complex situation, but as a static pattern, without the arrowheads, it does, I think, show the essential pattern of distribution of dominant families of mammals and birds in the Old World tropics and the north temperate zone now. It shows the relative richness and diversity of the tropical fauna and the absence of dominant families confined to the north temperate zone (see below). The arrowheads suggest how the pattern seems to have been formed.

I do not think Matthew's hypothetical pattern (6A) can be reconciled with the actual, existing pattern (6C, without the arrowheads), unless the existing pattern is abnormal, temporarily modified by special factors. Figure 6A puts too few dominant groups in the tropics and too many

in the north temperate zone. Additional old (stage 3) groups could be added to 6A to give the required diversity in the tropics (if the tropical fauna is mainly a huge accumulation of relicts while dominant animals evolve mainly in the much smaller and simpler north temperate fauna), but the dominant northern (stage 1) groups required by 6A do not seem to exist.

Where are the dominant groups of mammals and birds that, according to Matthew, should be evolving in the north temperate zone but have not yet spread into the tropics? This is a critical question. I want to answer it fairly, but the answer is partly a matter of personal judgment. Dominant groups are conspicuously successful ones. Existing, primarily north temperate families and subfamilies of land mammals (Darlington, 1957: 327) are Soricinae (red-toothed shrews), Talpidae (moles), Ochotonidae (pikas), Castoridae (beavers), Microtinae (field mice etc.), Zapodidae (jumping mice), Aplodontidae (sewellels), Spalacidae (mole rats), Seleviniidae (related to dormice), and Antilocapridae (pronghorns). Of these, only the Soricinae and Microtinae seem to me conspicuously successful, and they do not seem to be enough to satisfy the requirements of figure 6A. Existing, primarily north temperate families and subfamilies of land birds (*op. cit.*: 255) are Tetraoninae (grouse), Prunellidae (hedge sparrows), Bombycillinae (waxwings), and Certhiidae (creepers). These four groups total only 39 species. I do not consider any of them conspicuously successful. (Some families of far northern water birds are more successful.)

Can Matthew's dominant northern groups have been temporarily eliminated by special factors? I think not. They have hardly been destroyed by man; man has eliminated some mammals and birds in northern regions but many have survived, and the survivors should include the most dominant, evolving groups. They have hardly been destroyed by Pleistocene climatic fluctuations; many animals survived the Pleistocene north of the tropics, and evolving dominant mammals and birds should have been particularly able to survive. In fact, according to Matthew's thesis, conditions north of the tropics should be especially favorable to evolution of dominant groups now, for we are in a period of continental emergence and markedly zonal climate (1915, 1939: 7). Then, where are the dominant northern groups?

What does Matthew say about this? Not in *Climate and Evolution* but thirteen years later (1928: 84–85) he said, "The outstanding feature of the Holarctic fauna is that it includes today all of the most progressive and highly advanced groups of animals. Its animals are very distinctly at the top of the tree in their various branches. They are not always the largest of their kind but they have the most perfected mechanisms, the most finished adaptations for their several modes of life." Matthew did not consider the existing situation unfavorable. He thought evolution is proceeding on his pattern now, and that the best animals are all in the north temperate zone now. When he made the statement just quoted, he probably had in mind everything that had gone into *Climate and Evolution,* but here is the evidence he chose to cite (*loc. cit.*): "Rabbits are brought to Australia and flourish amazingly, at the expense of the native rabbit-like animals. Dogs are brought [to Australia] and almost wipe out the native dog-like carnivora. The mongoose is brought to Jamaica, nearly exterminates the few native mammals and makes heavy inroads on the birds. In most cases the introduction of Holarctic animals and plants into the southern continents and oceanic islands has wrought havoc with the native flora and fauna. . . ." This is all true, but Matthew deduced too much from it. The Holarctic Region, as Matthew himself defined it (*loc. cit.*), is essentially the world north of the tropics. The examples cited all compare Holarctic animals only with those of small, isolated continents and islands. These examples (and many others like them) show that

animals from the main part of the world are usually superior to those of smaller land masses, but they tell nothing about the relative dominances of north temperate and tropical animals within the main part of the world.

It seems to me that the pattern of distribution of dominant families in whole faunas of mammals and birds (fig. 6C) is inexplicable by Matthew's theory. He does not explain the pattern (he may not have known that it existed) but skips over it to compare Holarctic animals with those of smaller, isolated areas. But the pattern is explained if successive dominant families of mammals and birds have risen in the tropics and spread northward, and if some of them have later shrunk back into the tropics again. Exceptional groups have probably risen in the north and spread into the tropics, so that there has been an exchange. But figure 6C is close enough to the actual pattern of distribution of dominant families of mammals and birds in the main part of the Old World to suggest that the exchange has been very unequal, and that tropical origin and northward spread is the general rule.

Matthew's explanations. Matthew (1915, 1939: 7–8) thought that arid and cool phases of climate above the tropics would require animals to maintain themselves against inclemency of nature, scarcity of food, and variations of temperature, as well as against competition of rivals and attack of enemies, and that this would favor "greater activity and higher development of life" [i.e., general adaptation] as well as "special adaptations." He thought also that moist tropical climate, with abundant food, relatively constant temperature, and "larger percentage of carbonic acid and probably smaller percentage of oxygen in the atmosphere" would favor "sluggishness." And he thought that active, more highly developed northern animals would invade the tropics and compete with and replace sluggish tropical animals. These were "*a priori* deductions" to be tested against the evidence, especially against the record of mammals. Matthew's evidence seems at best inconclusive, as I have said. Are there other reasons to think that his climatic theory is right? Should northern climates offer a more effective challenge to evolving animals than, say, mass of life in the tropics? I do not know. If so, should not the most dominant animals evolve where the challenge of climate is greatest, in the arctic or in deserts? They do not seem to do so. Are northern animals really more active, tropical ones sluggish? They do not seem to be. Matthew's explanations, then, do not seem to agree with observed facts.

The Working Hypothesis

The relation of evolution and dispersal to climate is a complex and difficult subject, and not everyone will agree with my conclusions about it. Therefore, instead of stating fixed conclusions, I shall set up a working hypothesis which seems justified by the evidence, and then shall try to find what it implies and how it agrees with observable facts and evolutionary theory.

It is part of the hypothesis, justified by evidence, that area is one important factor in evolution of dominance and that, other things being equal, the most dominant animals usually evolve in the largest areas (p. 492). To this may now be added that climate is another important factor. Taken at face value, the evidence, especially the apparent histories of the best known and most recently dominant families of mammals and the pattern of distribution of dominant families of mammals and birds in the main part of the Old World (fig. 6C), justifies the hypothesis that the most dominant groups usually evolve in the Old World tropics and spread into the north temperate zone. Countermovements occur, but they seem to be relatively unimportant.

If evolving, dominant mammals and birds rise in the Old World tropics and spread northward, they follow the apparent pattern of cold-blooded vertebrates. The most dominant existing fresh-water fishes (cyprinids) and frogs (*Rana*) and some dominant genera of lizards and

snakes all extend from the Old World tropics far northward. Mammals and birds probably can spread northward more easily than cold-blooded vertebrates, but the pattern of movement seems the same. Why should it not be? Movement northward is, for all the groups, including the warm-blooded ones, from more favorable to less favorable climates and from more complex to simpler faunas (fig. 2). Why, then, should the warm-blooded groups reverse the pattern of evolution and movement of the cold-blooded ones?

Rising, spreading groups presumably continue to evolve, complexly, in the whole area they occupy. However, if the groups begin their rise in response to conditions in the Old World tropics, they would be expected usually to continue to evolve most effectively there, in continuing response to the same conditions. In this case, within each major group, successive dominant subgroups should rise in and spread from the Old World tropics.

Evolutionary Implications

Area, climate, and populations. Why should dominant animals evolve in the Old World tropics more than in other places? Apparently neither area alone nor climate alone can supply a satisfactory explanation. If area alone were involved, evolution should be as effective in the north temperate zone as in the Old World tropics. If climate alone were involved, evolution should be as effective in tropical America as in the Old World tropics. Apparently the effect of area and the effect of climate must be added together to make the Old World tropics a unique center of evolution of dominant vertebrates. The great area of Megagea (Africa + Eurasia + North America) apparently favors evolution of dominant groups. And so apparently does the warm, stable climate of the tropics. Where tropical climate is focused in a broad zone across Megagea, in the main part of the Old World, the effect of climate is added to that of area, making (apparently) optimum conditions for evolution of dominant animals.

How may area and climate exert effects on evolution?

Theoretically, warm, stable, tropical climate might accelerate evolution by increasing the rates of mutation or the numbers of generations of animals. But these effects are most likely to occur among cold-blooded animals and, I think, have not been observed among mammals and birds. Are there other ways in which both area and climate observably affect both cold-blooded and warm-blooded vertebrates so as to produce the requisite geographical pattern?

Area and climate might affect evolution through size of populations. Adaptation involves selection of advantageous mutations, and mutations occur in proportion to number of individuals in populations: the more individuals, the more (and sooner) the mutations, and the more rapid adaptation (including general adaptation) should be. If, therefore, large area and favorable climate increase the size of populations, that should accelerate general adaptation and the evolution of dominant animals. Or, area and climate might exert their effects through number of populations. If adaptation proceeds partly by selection of superior species—i.e., by selection of whole populations—it should be most rapid where populations are numerous as well as where they are large, for (other things being equal) the chance of superior populations appearing should be proportional to number of populations. Increase in number of populations might increase the force of selection, too. These are preliminary suggestions, to be considered in more detail in the following pages.

How are size of populations and number of populations related to area and climate?

Size of populations depends on area in some cases. Widely distributed populations on continents are obviously larger than populations confined to very small islands (even though the island popula-

tions may be denser), and this probably gives the continental populations a great advantage in adaptative evolution. But size of populations is not so simply correlated with climate. Large populations occur in some very adverse climates (hares and lemmings in the arctic are examples), and populations are not demonstrably larger in more favorable climates. In the tropics most animals seem to have small (sparse) populations. Some large populations do occur in the tropics, but whether they are larger than large populations in the north temperate zone is unknown. It is therefore impossible to say what role size of populations plays in the relation of evolution to climate. In earlier discussions (1948: 109–110; 1957: 565 ff.) I have probably overestimated its role.

Number of populations is correlated with both area and climate. To demonstrate the correlation with area we begin by comparing a very small island with a continent. If the island is small enough, it will have no vertebrates on it or perhaps a few small species but no large ones, while the continent will have many, diverse species. Then, comparing other islands of intermediate sizes, we find that there is no critical area below which faunas are limited and above which they are not. There is (other things being equal) an orderly relationship—the larger the area, the more numerous and more diverse the species in it. This relationship holds in a general way from the smallest island to the largest continent (Darlington, 1957: 482–483), although the effect of area on number of species is evidently modified by many other factors in particular cases.

The correlation between climate and number and diversity of species is similar. In the most adverse climates, say in the interior of Antarctica or on the Greenland ice cap, there are no vertebrates except passing birds. In less extreme parts of the arctic there are small numbers of species. In temperate, seasonal climates species are more numerous. And in warm, relatively stable, tropical climates they are still more numerous, and more diverse. There is (other things being equal) an orderly relationship here, like but more complex than the relationship of area to number and diversity of species—the warmer and more stable the climate the more and more diverse the species. This is true even of mammals (fig. 2) and birds, which are much more numerous (in species) and more diverse (in ancestry and relationships) in the tropics than elsewhere.

Area and climate, then, have similar effects on number of species—or of populations—and they reinforce each other. The largest and most diverse faunas—the most populations—are in the largest areas and the warmest, most stable climates.

The observed general relation of area and climate to number of populations is mapped diagrammatically in figure 7. The three principal land masses of the world are represented in proportion to their areas. Each mass is divided into squares according to its area: in Megagea (area 10A) the squares are double the dimensions of those in Australia (area 1A), and in South America (area 3A) the squares are intermediate. And within each mass the squares in the tropics are double the dimensions of those in the temperate zone. I have made the diagram in this way because multiplication of area by 10 does double number of species in some cases (e.g. species of reptiles and amphibians on some islands in the West Indies, Darlington, 1957: 483–484), and because number of species is sometimes approximately doubled in tropical as compared with temperate regions (e.g. species of mammals in tropical as compared with temperate eastern Asia, *op. cit.*: 328). As thus drawn, the sides of the squares are VERY ROUGHLY proportional to the numbers of populations to be expected in different places, if my assumptions are correct. Moreover if the complexity of interaction of populations increases exponentially with their number (as suggested by Prof. E. O. Wilson, in conversa-

tion), the areas rather than the sides of the squares may be VERY ROUGHLY proportional to the complexity (and force?) of selection in different places.

Arrows have been added to the diagram (fig. 7) to suggest VERY ROUGHLY the relative amounts of movement in different directions that might occur if area and climate determine numbers of populations, and if numbers of populations determine the effectiveness of adaptive evolution. The arrows make a world-wide pattern that agrees reasonably well with the apparent main pattern of dispersal of dominant vertebrates (fig. 1, B) (Darlington, 1957: 571).

This diagram (fig. 7) is extremely oversimplified. Rainfall is ignored, and the distribution of temperature is much simplified. Habitat is ignored; the area available to different animals in different regions must depend partly on whether they live in forests or steppes or deserts, and number of populations must vary with continuity or discontinuity of habitats; but habitat restrictions may be partly overcome by ecological radiations of dominant animals. Partial or intermittent barriers (e.g. Bering Strait) are ignored, although they must often reduce or divide populations. The structure of populations is ignored; it is assumed that each species is

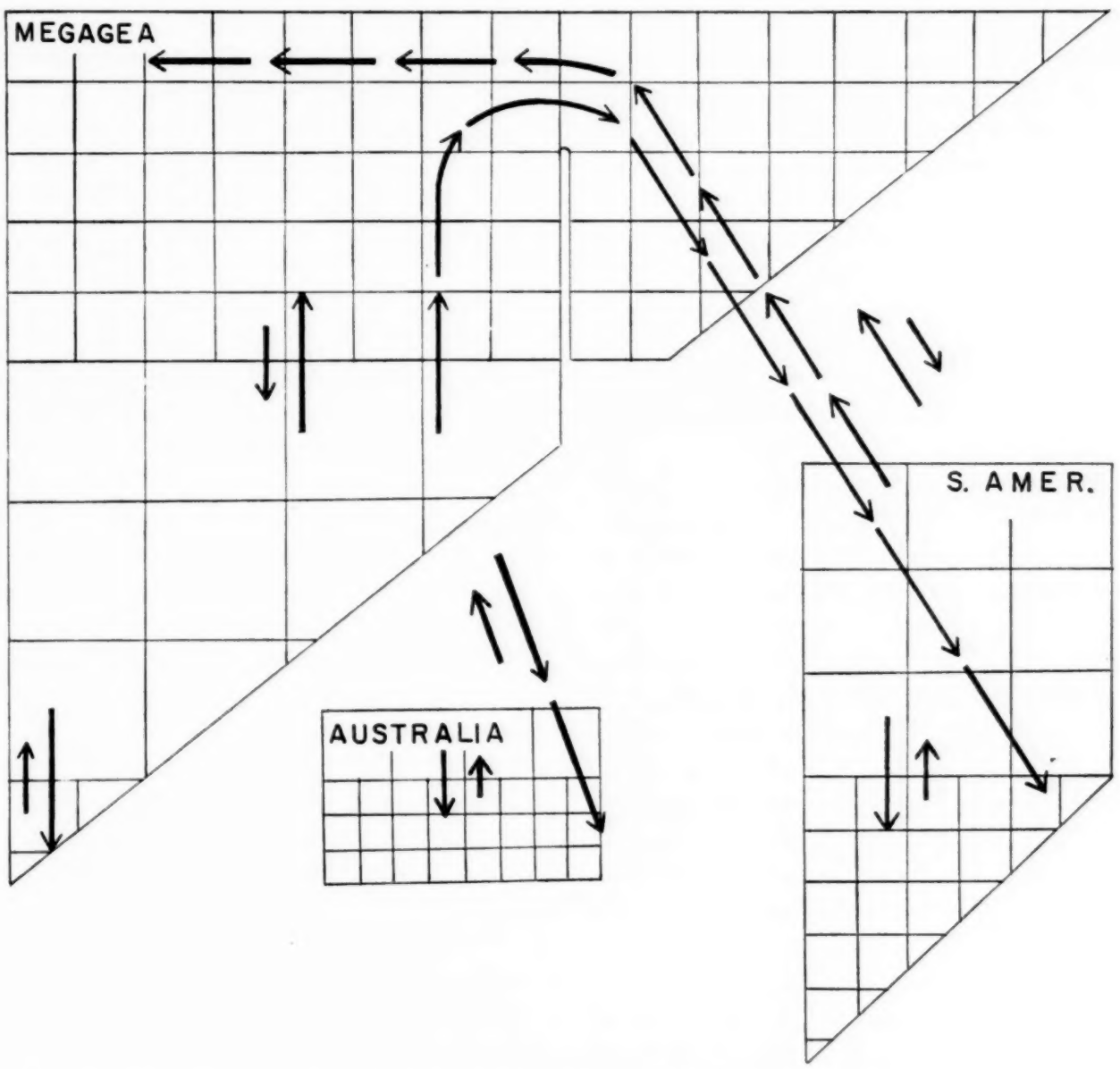


FIG. 7. Map-diagram of suggested relation of area and climate to number of populations and to evolution and dispersal of dominant vertebrates. See text for method of construction. Length of arrows is (very roughly) proportional to amount of movement expected in directions indicated.

one population, but some species in some places (especially in large areas or discontinuous habitats) probably consist of many separate subpopulations, and this probably affects evolution. And, perhaps most important of all, figure 7 ignores the effect of time. Under special conditions, when large faunas become isolated on small islands, time may reduce the number of species. More often, time probably increases the number. If, for example, there are more species of birds in a given area in tropical South America than in tropical Asia, this may be partly because species of birds have been multiplying in South America for a longer time without major replacements.

Nevertheless figure 7 gives a pattern which agrees in general with the apparent pattern of dispersal of dominant vertebrates and which is based on the observed general relation of number of species to area and climate. I can see no other way of deriving the requisite pattern from observed facts. I think, therefore, that the diagram is probably somewhere near the truth, in a very oversimplified way. At least it provides a further working hypothesis by which to examine general adaptation.

The diagram adds the effect of climate to the effect of whole area. This is necessary to make a pattern that conforms to the apparent pattern of vertebrate dispersal, with one main center, in the Old World tropics (p. 503). But the effect of climate can be added to the effect of whole area only if, besides much movement out of the tropics, there is some movement back into them from other parts of the whole area: the whole of Megagea must produce moderately dominant groups some of which then move into the tropics and are raised to predominance there. Counter-movements like that of the horses, then, are not only observed, and expected from what is known of the nature of complex faunal movements (p. 499), but apparently must occur to make the Old World tropics the main center of evolution of dominant vertebrates.

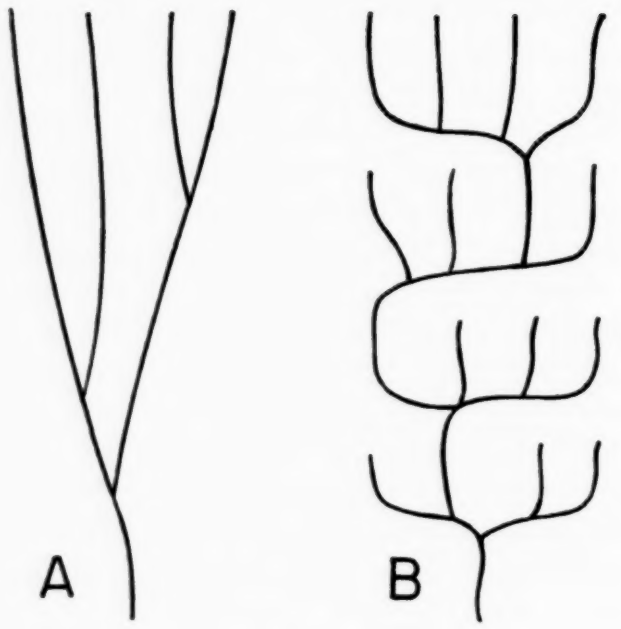


FIG. 8. Diagrams of evolution of four species: A, without selection of populations; B, with repeated selection and re-radiation of populations.

Populations, faunas, and evolution. Large populations probably have an adaptive advantage everywhere (p. 503). The diagram (fig. 7) implies and requires that large number of populations gives an additional, perhaps greater evolutionary advantage. Mass and diversity of populations might increase selective pressures on individuals within populations. Or populations might be selected as wholes, with continual radiations and reradiations of successive, selected, superior populations, extinction of others, continual replacement, and continual movement (spreading) of the superior, evolving populations (fig. 8). That dominant animals often do evolve in this way, with continual replacement and much movement, is suggested by the present distributions of many animals and by the fossil records of the best known groups. The history of man during the last few thousand years exemplifies evolution with continual replacement and much movement of populations.

Evolution by selection of whole populations may be more efficient than evolution by selection of individuals in single populations, for two reasons. First, although selection may proceed in many different directions in one population at one time

(e.g. toward concealing coloration, better teeth, and increased intelligence) without the different selective progressions interfering much with each other, interference probably does occur in some cases. For example sexual selection may interfere with or oppose non-sexual selective processes and may produce, often in one sex only, characters that give some individuals an advantage in mating but that are otherwise disadvantageous to the species. In such cases there may be an opposition of interests between individual animals and the species they belong to: what is good (or selectively advantageous) for individuals may be bad for the species. This may be true of increase of size. Large size may give individuals an advantage in many ways but may impose an evolutionary disadvantage on species in the course of time (see below). Evolution of single species is therefore likely to be a compromise between what is good for individuals and what is good for the species, and evolution of single species is likely to be retarded or deflected accordingly. But selection among species—selection of whole populations—involves less compromise and is more likely to be simply selection of the most efficient animals. Selection of species (or whole populations) may therefore be more efficient than selection of individuals within species, so far as general adaptation—evolution of generally better animals—is concerned.

The second way in which selection of whole populations may be more efficient than selection of individuals in single populations depends on the nature of the evolutionary process. Evolution involves changes of gene systems by mutation, recombination, and selection. Recombination is very important. Its evolutionary advantage is evidently so great that it more than outweighs the wastefulness of sexual reproduction, which often halves the number of individuals that bear offspring but makes recombination of genes possible. The great importance of recombination is confirmed by mathematics. Potential recombinations of genes give all ordinary species a great "store of variability" sufficient for varied and extensive evolutionary modifications without the occurrence of any new mutations (Fisher, 1930: 95–96; Wright, 1949). Only a comparatively small amount of recombination-variability is available in a single, random-breeding population. More becomes available if the population is divided into subpopulations which are partly isolated from each other but which interbreed occasionally. Such subpopulations may evolve divergently, by recombination of genes, and subpopulations may then be selected almost as wholes. The most favorable situation may well be where many widely distributed but very sparse populations occur together, each tending to form many small, partly isolated subpopulations. Under these conditions a maximum of recombination-variability should occur, and selection both of subpopulations and of whole populations can occur, with extinctions and replacements at both levels, and this should result in much more efficient use of recombination-variability, and more efficient adaptation, than can occur in single populations. These conditions may be approximated in large, widely distributed faunas in the tropics.

Under these conditions, the size of individual animals might have a profound effect on evolution. Among given animals during limited times large size may be advantageous in competition for mates and in other competition among individuals of one species and (less often ?) among different species, and size may increase progressively by selection. But increase of size increases the requirements of each individual and reduces the size and/or number of populations that can exist in a given area and climate. Moreover, since large vertebrates can move farther than small ones, increase of size of individuals may tend to make populations more uniform, less likely to become divided into partly isolated subpopulations. These changes toward smaller,

fewer, less subdivided populations would presumably reduce the efficiency of adaptive evolution. And, eventually, the advantage of large size might be overcome by the more efficient evolution (general adaptation) of smaller animals. This might give a basis for evolution of successive groups, each group going through a cycle of radiation, increase of size, and then extinction of the large forms, followed by re-radiation of new, smaller forms, which in turn would increase in size, etc. Successions something like this, but irregular and very complex, seem to have occurred during the evolution of terrestrial vertebrates.

The relation of number and structure of populations to evolution can be approached in another way. Evolution presumably makes situations favorable to itself: effectively evolving populations are presumably selected, and combinations of populations favorable to evolution are presumably selected too. This kind of selection is complex and presumably requires much time and also a fairly stable climate, for catastrophic changes would destroy evolving combinations. We might therefore expect to find populations and combinations of populations favorable to effective evolution in the relatively stable tropics rather than the unstable north temperate zone. This is what we do seem to find, as I have tried to show in the preceding pages.

Methods; Restatement of the Hypothesis

I first wrote this paper as a sequence of evidence, arguments, and "proved" conclusions, but then it read as if I had taken an arbitrary position and were defending it. This was of course the case, and it inevitably still is the case to some extent. However I have tried to limit this aspect of the paper by partly changing the method of presentation. I have had to present facts and situations as they seem to me to be, and say what they seem to me to mean. But then, instead of trying to prove conclusions, I have set up a working hypothesis based on evidence taken at face value, and I have tried to test it and explain it by means of observable facts and relationships, so far as possible. This method may help to reduce personal bias, may prepare the way for experimental and mathematical treatment of the observed relationships, and may lead toward the truth more surely than personal judgment would. The change in method has led to two significant changes in my original conclusions: to stressing unequal exchange rather than one-way movement between the Old World tropics and the north temperate zone, and to stressing number rather than size of populations in relation to evolution. The latter change brought my hypothesis more into line with the expectations of mathematical evolutionists.

Restated very briefly, the hypothesis is this. The most dominant vertebrates usually evolve in the Old World tropics and spread northward, but some countermovements do and must occur. Both area and climate contribute to making the Old World tropics a unique evolutionary center: moderately dominant groups evolve in the great area of Megagea, and are then raised to predominance in the Old World tropics. Both area and climate are observably correlated with number of populations: the larger the area and the more favorable the climate, the more and the more diverse the populations. Large number of populations may accelerate adaptation by selection of whole populations, which should tend to avoid individual-versus-species oppositions and make effective use of recombination variability. The situation in the tropics, of many, sparse, perhaps subdivided populations, may be, and logically ought to be, most favorable to efficient selection and adaptation, including general adaptation.

This hypothesis is very much oversimplified. It stresses only one (but perhaps the most important) of many factors that probably affect the evolution of different animals in different places.

Postscript

G. E. Hutchinson's "Homage to Santa Rosalia or Why Are There So Many Kinds of Animals?" (1959, *American Naturalist,* **93**: 145–159) and William L. Brown Jr.'s "General Adaptation and Evolution" (1959, *Systematic Zoology,* **7**: 157–168), both concerned with some aspects of evolution dealt with here, appeared while this paper was in press. Also while this paper was in press I have reread "Evolution in the Tropics" (1950, *American Scientist,* **38**: 209–221) by Theodosius Dobzhansky. He suggests that selection is controlled in the cold zone mainly by the physical environment and in the tropics mainly by the more complex biological environment, and that the latter favors evolution of new modes of life and more advanced types of organization. It would be interesting to see this idea developed in detail.

Acknowledgments

I am indebted to Dr. William L. Brown Jr. and Prof. Edward O. Wilson for reading the manuscript of this paper and for a number of useful suggestions.

Summary

(1) This paper is concerned with general adaptation, which is the kind of evolution that produces dominant animals (p. 488).

(2) Area is one important factor in evolution of dominance: the most dominant animals seem usually to evolve in the largest favorable areas (pp. 490–492). How climate affects evolution is the question (fig. 2).

(3) The apparent histories of the best known and most recently dominant groups of mammals and the distribution of dominant families of mammals and birds in the Old World tropics and the north temperate zone (fig. 6C) indicate that the most dominant warm-blooded (like cold-blooded) vertebrates usually evolve in the Old World tropics and spread northward, although some countermovements occur (pp. 495–502, summarized p. 502).

(4) Apparently neither area alone nor climate alone can make the Old World tropics a unique evolutionary center. The effects of area and climate must be added together (p. 503).

(5) Tropical climate might accelerate mutation or reproduction and thus accelerate evolution; but this is unlikely among warm-blooded animals. Large populations presumably have an advantage in evolution by mutation and selection of individuals; but, although large populations occur in large areas, they do not seem to be especially characteristic of tropical climate. Number of populations of both cold- and warm-blooded vertebrates is correlated with both area and climate: the larger the area and the warmer and more stable the climate, the more and more diverse the populations (pp. 503–504).

(6) If the relation of area and climate to number of populations is mapped diagrammatically (fig. 7), a world-wide pattern is formed, centered on the Old World tropics, which fits the apparent geographical pattern of evolution and dispersal of dominant vertebrates (fig. 1, B) (pp. 504–506).

(7) This suggests that effective evolution—general adaptation—is correlated with number of populations and occurs partly by selection of whole populations, with continual extinctions, replacements, and movements (spreadings) of evolving populations (fig. 8). There are indications that dominant groups do evolve in this way (p. 507).

(8) Selection of whole populations tends to avoid individual-versus-species oppositions and makes effective use of recombination-variability. The situation in the tropics—occurrence of many, sparse, perhaps subdivided populations—may be especially favorable to selection of populations and use of recombination-variability. Evolution ought to make situations favorable to itself in the stable tropics (pp. 507–508).

(9) Conclusions are presented as a working hypothesis, justified by evidence taken at face value, based as far as possible on observable facts and relationships, and capable of experimental and mathematical testing.

LITERATURE CITED

AUBERT DE LA RÜE, E., F. BOURLIÈRE, AND J.-P. HARROY. 1957. The Tropics. New York, Alfred A. Knopf.

DARLINGTON, P. J., JR. 1948. The geographical distribution of cold-blooded vertebrates. Quart. Rev. Biol., **23**: 1–26, 105–123.

——. 1957. Zoogeography, . . . New York, John Wiley; London, Chapman & Hall.

——. 1959. Darwin and zoogeography. Proc. American Phil. Soc., **103**: 307–319.

DARWIN, C. (1859) 1950. On the Origin of Species . . . [reprint of the first ed.]. London, Watts & Co.

FISHER, R. A. 1930. The Genetical Theory of Natural Selection. Oxford, Clarendon Press.

HOPWOOD, A. T. 1954. Notes on the recent and fossil mammalian faunas of Africa. Proc. Linn. Soc. London, **165**: 46–49.

LEAKEY, L. S. B., AND W. E. LE GROS CLARK. 1955. British-Kenya Miocene expeditions. Interim report. Nature, **175**: 234.

MATTHEW, W. D. (1915) 1939. Climate and evolution [reprint]. Special pub. New York Acad. Sci., **1**.

——. 1928. Outline and general principles of the history of life. Univ. California Syllabus Ser., No. 213.

SIMPSON, G. G. 1940. Mammals and land bridges. J. Washington Acad. Sci., **30**: 137–163.

——. 1945. The principles of classification and a classification of mammals. Bull. American Mus. Nat. Hist., **85**.

——. 1947. Evolution, interchange, and resemblance of the North American and Eurasian Cenozoic mammalian faunas. EVOLUTION, **1**: 218–220.

WRIGHT, S. 1949. Adaptation and selection. (In) Jepsen *et al.*, Genetics, Paleontology, and Evolution: 365–388. Oxford, Clarendon Press.

DELPHINIUM GYPSOPHILUM, A DIPLOID SPECIES OF HYBRID ORIGIN

HARLAN LEWIS AND CARL EPLING

Department of Botany, University of California, Los Angeles

Received February 11, 1959

The purpose of this paper is to examine evidence that *Delphinium gypsophilum* had its origin by hybridization at the diploid level without change in chromosome number and by a more direct process of recombination than that ordinarily referred to as introgression. This hypothesis was first stated in an abstract (Lewis and Epling, 1946), and was discussed later by Epling (1947) and by Stebbins (1950) after more data were at hand. We shall now discuss the total evidence that has accrued since our abstract appeared.

The genus *Delphinium* is mostly associated with plant communities that have been derived during later Cenozoic time from the Arcto-Tertiary Geoflora of the New and Old Worlds. The species we have studied are associated with present derivatives of the Madro-Tertiary Geoflora: the oak woodland, chaparral, and to some extent, the shrubby communities of the Sonoran desert. They have generally independent ranges with a considerable overlap in some cases, and some are included within the ranges of others. Whether their ranges overlap or are included, the species that grow in the same vicinity generally occupy ecologically different sites. Repeated observations indicate a considerable restriction of migration from colony to colony, either by pollen or by seeds. For example, blue or white flowered colonies of the same species persist unchanged for indefinite periods within short distances of each other and in similar habitats (Epling and Lewis, 1952). Thus, each colony has a considerable permanence of its adaptive attributes.

The colonies of two species are sometimes contiguous and may be composite, providing ample opportunity in either case for gene exchange between them. The vectors of pollen are bees, especially *Bombus*. There is no apparent selectivity on their part for flower color or conformation, and visits from plant to plant in mixed populations appear to be at random. The species with which we shall deal are self compatible, but the behavior of the bees results mostly in cross pollination because of protandry. Barriers to interspecific hybridization are often lacking, not only because of the pollinating process, but also for genetic causes. A full seed set occurs after most first crosses. The seeds are viable for the most part and generally produce normally developed and vigorous F_1 plants. The fertility of F_1's when selfed is sometimes high and sometimes low but is reasonably consistent for each interspecific cross. The seed set is generally higher after backcrossing. Even though fertility is low for a particular cross, some individuals, even triploids, will set occasional viable seeds.

With these opportunities for gene exchange one might expect to find hybrid swarms wherever two species intermix. This is seldom the case. On the contrary, hybrid individuals in flower are few and seldom seen. Mixed colonies are mostly divisible into two taxa of which neither differs substantially from its relatives throughout their main ranges. Nevertheless, a considerable number of hybrid plants may be present in the vegetative state in these colonies and reach flowering only in response to particular conditions (Epling and Lewis, 1952). These hybrids can and do persist without flower-

ing for many years in virtue of root dormancy by which the adaptive potentials that each genotype carries are stored, so to say, against the rainy day when each may flower and fruit.

This dormancy is a characteristic of the *Delphinium* species that occur in Madro-Tertiary environments. It rests on the capacity of the roots to become dormant and remain so for an indefinite period during which they are brittle, chalky within, and apparently lifeless. With the onset of dormancy, the flowering shoot dies back to the crown regardless of whether the flowers or seeds have matured. This response is induced by periods of seasonal drought that characterize these environments. With the advent of the winter rains the roots again become fleshy and active. Whether a rosette of leaves will form at the crown and how long it will function before withering; whether a flowering shoot will form and remain alive long enough to mature the seeds; whether the seeds will germinate during the following winter or remain dormant in the soil: all these apparently depend on the responses of individual genotypes to particular environments. Resumption of the yearly cycle of development is seemingly invoked by the conditions of weather acting on each local soil type. The result is that a flowering colony will consist in one year of hundreds or even thousands of individuals, and in the following year it may have only tens or perhaps none. In some years, some species, such as *D. gypsophilum,* seem to be geographically continuous in their areas even though locally concentrated. The spacing and grouping of individuals from year to year also varies (Epling and Lewis, 1952). The effect of root dormancy on these differences of population structure and density and gene exchange is crucial for understanding the dynamics of a mixed population. This effect would be particularly important for the establishment of genetic variants that might be harbored in the colony for long periods, especially when one takes into account the more or less rhythmic long range fluctuations of climate which may become critical for the persistence of taxa in the Madro-Tertiary environments they occupy.

Comparison of the Species

We shall be primarily concerned in this discussion with three species: *D. recurvatum, D. hesperium pallescens* and *D. gypsophilum* (Lewis and Epling, 1954). We shall refer to the second simply as *D. hesperium.* All are diploid (n = 8) but *D. gypsophilum* has a tetraploid race that is indistinguishable from the diploid in external morphology. We shall refer only to the diploid.

These taxa grow primarily in the foothills that border the western and southern parts of the central valley of California (fig. 1). Their ecological preferences are as readily distinguished as their morphology. *Delphinium recurvatum* occurs chiefly in saline areas on the lower slopes of the foothills that are dominated by *Atriplex,* and extends into the valley itself along salty streams in association with *Allenrolfia.* It occupies one of the most drastic and arid habitats, climatically and physiologically, to which the genus is adapted. It is closely related to, but distinct from, a desert species, *D. parishii,* to which we shall refer. *Delphinium hesperium,* on the other hand, occupies the oak woodland which is the most mesic of present day derivatives of the Madro-Tertiary Geoflora in this region. This woodland occurs on the higher, well drained slopes that surround the valley. Because of topography it frequently approaches the valley bottom. In the western foothills it consists chiefly of *Quercus douglasii* associated locally with occasional individuals or small groves of *Q. lobata. D. gypsophilum* occupies a habitat that is intermediate between these two extremes. It is abundantly distributed throughout a narrow zone of grassland along the western and southern borders of the valley. The original cover of this

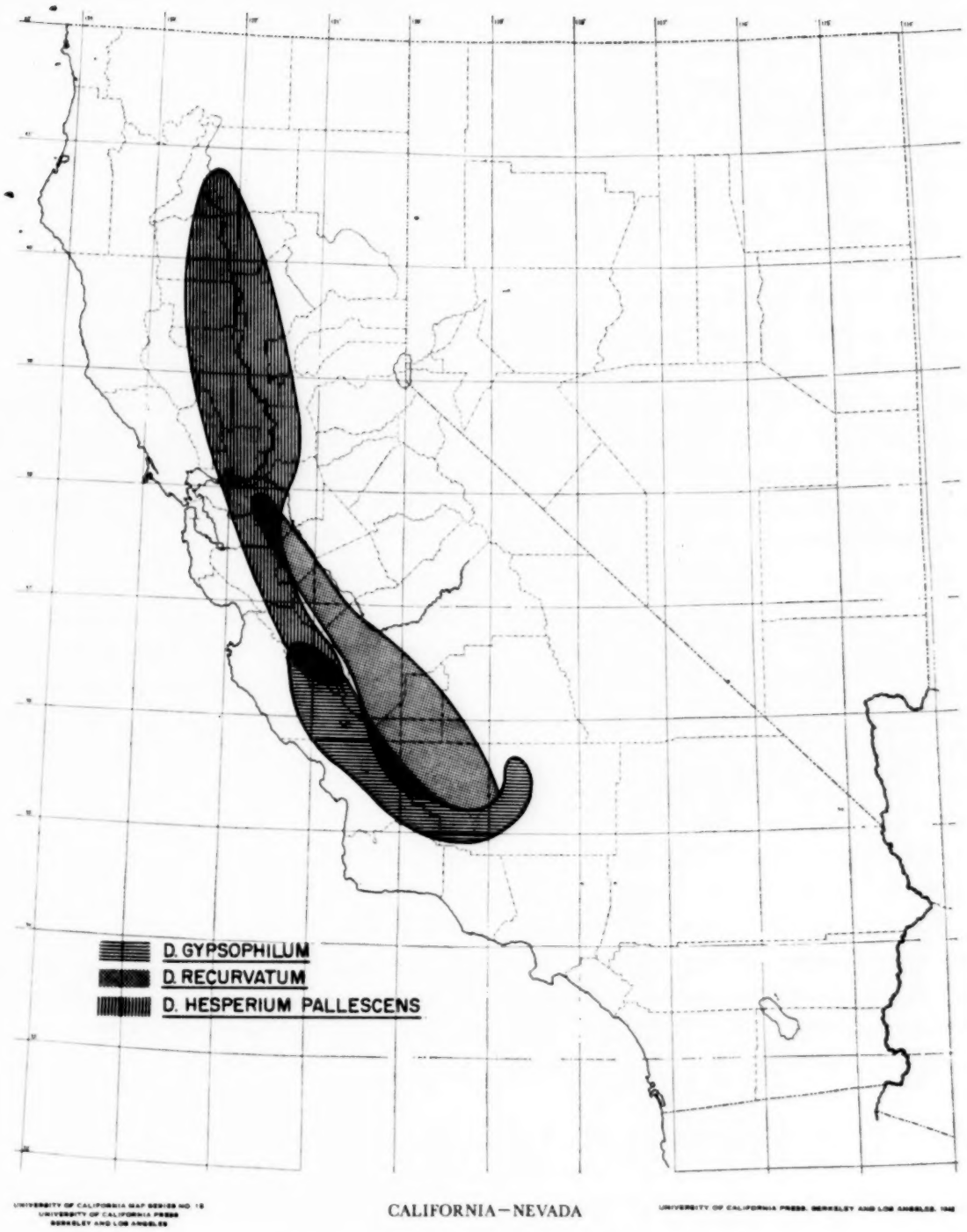


FIG. 1. Approximate geographic range of the two probable parental species and their hybrid species, *D. gypsophilum*.

zone was doubtless bunch grass, dominated by species of *Stipa*, together with many small perennial herbs and annuals. The perennial grasses are now largely replaced by mediterranean annuals, in genera such as *Avena* and *Bromus*.

There can be no doubt that the habitats of the species of *Delphinium* we shall refer to have been modified by man's occupation during the last century, particularly by grazing. Thus, many colonies of *D. hesperium* are found only along roads outside of fences and not so much in the grazed woodland within them. We have estimated that some of the individuals we have found may be fifty years old or more, suggesting that these present limited habitats probably represent their original ranges fairly well. At their closest points of contact, *D. hesperium* and *D. recurvatum* are separated now by several miles and by a considerable ecological barrier. This does not preclude the possibility that they were once within pollinating range during recent time. Both species overlap at some points with *D. gypsophilum*.

D. recurvatum, D. gypsophilum and *D. hesperium* are instantly determinable throughout their respective ranges by the summation of characters contrasted in table 1. Most of these characters are complex, such as leaf habit and the conformation of the flower. Taken individually, their expression overlaps in the different species, but the modes are quite distinct. We have been able to treat quantitatively some other characteristics, such as internode number and flower number; but they also overlap, partly because of individual responses in development. Others, such as the presence or absence of pubescence or glaucousness, and the nature of the seed coat, are sharply differentiated in both wild and cultivated specimens. All are determined by multiple genetic factors, and to judge from segregations in F_2 progenies are more or less independently determined. However, glabrousness and glaucousness, on the one hand, and pubescence and nonglaucousness, on the other (the first being a characteristic of *D. recurvatum* and *D. gypsophilum,* the second of *D. hesperium*), are linked to an appreciable extent, sufficient for them to show strong positive correlations in segregation. We have not been able to measure linkage precisely because of the high frequency of gametic and zygotic elimination of genotypes and our inability to determine the degree of its randomness with respect to any given phenotypic expression.

The associations of characters represented in each column of table 1 are the result of careful analysis of hundreds of individuals, both wild and cultivated. The fairly uniform association of these characters over wide areas that are ecologically differentiated, and the probable persistence of these associations for the long period of geological time that their distribution suggests, argue strongly that each species is genetically integrated throughout its range. Each species is a well defined mode of evolution. The contrast between each character of *D. recurvatum* and *D. hesperium* is sharp, but it will be noted that the characters of *D. gypsophilum* are a composite; some of its attributes resemble the corresponding ones of *D. recurvatum,* some are intermediate, and some resemble those of *D. hesperium.* The first point of our argument is that, taken as a whole, *D. gypsophilum* is intermediate between *D. recurvatum* and *D. hesperium* in its character associations (fig. 2).

The differentiating traits of each spe-

TABLE 1. *Comparison of the more conspicuous morphological traits of* D. gypsophilum *with those of* D. hesperium pallescens *and* D. recurvatum

	D. hesperium pallescens	*D. gypsophilum*	*D. recurvatum*
plant height	tall	tall	short
no. of internodes between inflorescence and rosette	many	many	few
stem pubescence	puberulent	glabrous	glabrous
stem surface	non-glaucous	glaucous	glaucous
stem color	green	intermediate	red
stem thickness	stout	stout	slender
leaf segments	broad	intermediate	narrow
leaf venation	prominent	inconspicuous	inconspicuous
inflorescence	dense	intermediate	lax
flower number	many	many	few
flower conformation	cupped	rotate	reflexed
sepal color	white	white	lavender
sepal shape	broad	intermediate	narrow
seed coat	adhering	loose	loose

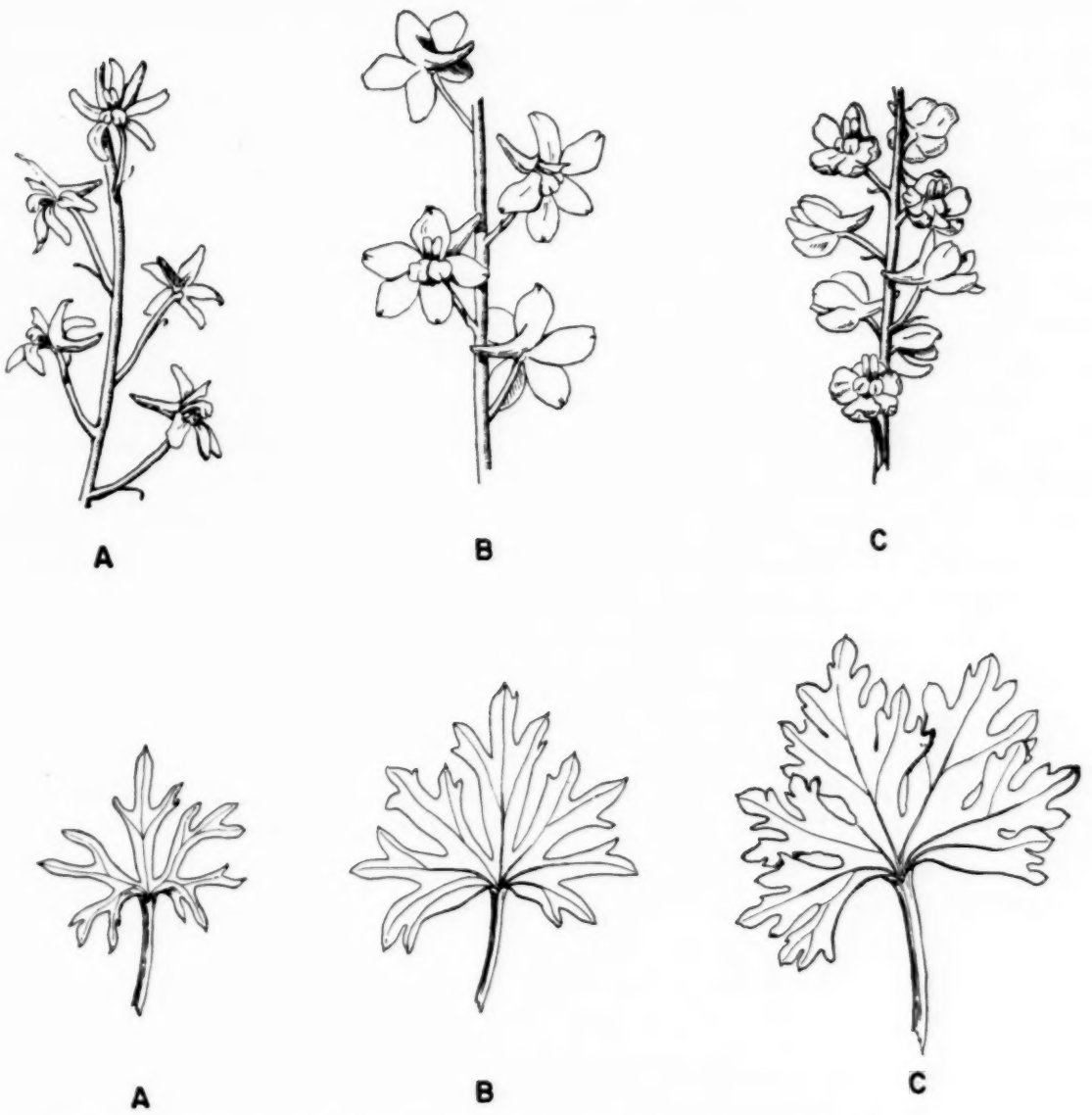


FIG. 2. Outline drawings of the habit of flower, inflorescence and leaf of A) *D. recurvatum*, B) *D. gypsophilum*, and C) *D. hesperium pallescens*.

cies vary to some extent from colony to colony. Two examples will illustrate the scope of this variance. The leaf segments of *D. recurvatum* from population 542 (table 2) when grown under uniform garden conditions, were consistently broader and fewer in number than those of population 413, when both were compared in a given year. In the same manner, plants of *D. gypsophilum* from population 545 were stockier than those of population 411. The wild colonies from which such transplant populations were made are generally fairly uniform; but the progenies from selfing these transplants are usually more variable than the colony from which their parents were obtained. This would be expected of a highly heterozygous normally outcrossed species when grown under less rigorous conditions than prevail in the wild.

CULTURE AND SCORING

Stocks to be used as parents for experimental crosses were transplanted from wild colonies (table 2). The transplants were made sometimes during the growing period and sometimes after the plants had become dormant. Transplants were grown in 4-inch pots sunk in wood shavings out of doors. When in bud or flower they and experimental hybrids were moved into an insect free screenhouse in which the pollinations were made. Flowers to be crossed were emasculated before the stigmas became receptive. In crossing hybrid and nonhybrid individuals, the hybrid was used as the female

parent unless otherwise indicated. The seeds set by each cross were counted and sown in 4-inch pots in the autumn. Germination frequencies were obtained the following spring by counting the seedlings in each pot. Those desired for further study were allowed to become dormant and were separated and planted in individual pots the following autumn prior to the winter rains. Because of small size, yearlings were seldom scored or used for hybridization. Normal size is attained in the second year during which most crosses were made and the characters scored. These included the size and conformation of the uppermost rosette leaf, the amount and kind of pubescence on the leaves and stem, the presence or absence and distribution of glaucousness on the stem, the number of flowers, the size, shape, and color of the sepals, and the conformation of the flower. Color was recorded by using *A Dictionary of Color* (Maerz and Paul, 1930).

Fertility was measured primarily by the proportion of seed set per follicle and the frequency of germination (tables 3 and 4). We also ascertained the proportion of visibly good pollen, but found it of little use in estimating fertility because the percentage of good pollen is highly variable, even among individual wild transplants. Most individuals of *D. recurvatum* and *D. hesperium pallescens* have better than 75 per cent good pollen, but some may be as low as 30–35 per cent.

TABLE 2. *Collections of* Delphinium gypsophilum (*diploid only*), D. hesperium pallescens, *and* D. recurvatum *transplanted to the experimental garden and used as parents for hybridization studies. All localities are in California*

	Accession number	Locality	Collector	Date
D. gypsophilum	411	1 mile north of McKittrick, Kern County	Mehlquist	1940
	431	10 miles east of King City, Monterey County	Lewis and Mehlquist	1941
	531	U. S. Highway 399, 5 miles south of Maricopa, Kern Co.	Epling and Lewis	1944
	545	Same locality	Epling and Miles	1945
D. hesperium pallescens	444	Marsh Creek Road, 2.3 miles west of Byron, Contra Costa County	Lewis and Mehlquist	1942
	445	East margin of Antioch Golf Course, Contra Costa County	Lewis and Mehlquist	1942
	446	5.4 miles north of Marsh Creek road on the road to Antioch, Contra Costa County	Lewis and Mehlquist	1942
	449	2.8 miles south of the entrance to Pinnacles National Monument, San Benito County	Lewis and Mehlquist	1942
	450	0.5 mile south of Paicines, San Benito County	Lewis and Mehlquist	1942
	586	1.0 mile south of Marsh Creek Road on the road to Livermore, Contra Costa County	Epling	1945
	589	Same locality	Epling and Lewis	1945
D. recurvatum	412	7.2 miles north of Coalinga, Fresno County	Mehlquist	1941
	413	13.6 miles north of Coalinga, Fresno County	Mehlquist	1941
	453	Same locality as 412	Lewis and Mehlquist	1942
	542	5 miles west of State Highway 120 on the road to Byron Hot Springs	Epling and Miles	1945

TABLE 3. *Seed set and germination of the* Delphinium *crosses indicated.* Female parent is listed first

	Number of combinations	Mean seed set per follicle	Number of seeds planted	Number germinated	Per cent germination
hesperium × *hesperium*	14	15.3	707	405	57
hesperium × self	23	14.7	1578	508	32
hesperium × *recurvatum*	6	5.2	295	143	48
recurvatum × *recurvatum*	19	16.7	1029	570	55
recurvatum × self	11	17.8	1528	511	33
recurvatum × *hesperium*	6	15.2	615	231	38
recurvatum × *gypsophilum*	5	13.4	125	87	70
gypsophilum × self	6	24.4	630	258	43
gypsophilum × *hesperium*	1	22.0	25	15	60
gypsophilum × *recurvatum*	1	35.0	25	14	56

The scoring of traits in successive years is influenced by environmental modifications and comparisons between parents and their progeny is open to error for this reason. Even though the conditions of culture are "uniform," the weather differs from year to year and the composition of the soil in the pots changes. Thus, it is not surprising that some traits chosen for study have been found to vary to some extent from year to year. For example, the uppermost rosette leaves on a given plant of *D. variegatum* may show greater variation in shape from one year to the next, than the range in one year of variation of the entire sample (Lewis, 1947). Comparisons of vegetative traits of this sort must, therefore, be treated with caution. Those we have made for the purposes of this paper have been drawn from contemporary progenies as far as possible.

FERTILITY

We have estimated fertility within and between species by the number of seeds set per follicle, of which there are three in each flower, and the germination of each class in the following spring after they were exposed in four inch pots to the prevailing temperatures and the winter rains. The frequencies of germination were reasonably consistent and representative although they may not represent the total capacity of the seeds to germinate, inasmuch as an occasional seed was found to hold over until the following year, perhaps because of a different reaction to external conditions. Most follicles in nature are well filled and the normal seed numbers for each species are about the same as those shown in the second column of table 3, where wild transplants were crossed or selfed. The seed set of *D. gypsophilum* is definitely higher than that of *D. recurvatum* or *D. hesperium*. Seed set in the F_1 crosses of the species we have worked with, including those listed above, generally approximates that of the parental species. A noteworthy reduction was found, however, in the initial cross between *D. recurvatum* and *D. hesperium* when the latter was used as the female parent. Seed germination of the crosses in either direction was not substantially different, but was somewhat lower than that of the wild parents.

Regardless of the direction of the cross, the fertility of the selfed or backcrossed F_1 hybrids between *D. recurvatum* and *D. hesperium* is low, compared with the normal seed set of the species or with that of the first crosses (table 4). The seed set from backcrossing was higher than from selfing, and seed germination was much higher. But of particular interest, the cross of *D. gypsophilum* to the

F_1 was higher on the average, than backcrosses to either parent. In contrast to the evident reproductive barrier between *D. recurvatum* and *D. hesperium, D. recurvatum* and *D. gypsophilum* are highly interfertile. This sharp difference is probably caused primarily by the different chromosome architecture of the species involved.

The haploid chromosome complement of *Delphinium* consists of 8 chromosomes including two long metacentrics, five shorter subequal acrocentrics and a shorter metacentric (Lewis *et al.*, 1951). We have found no evidence of structural heterozygosity in wild individuals of either *D. recurvatum* or *D. gypsophilum,* nor in the F_1 hybrids from crossing different transplants of these species. The case of *D. hesperium* is different. In the first place, it is itself structurally variable and second, F_1 hybrids we produced between *D. hesperium* and *D. recurvatum* were heterozygous for at least two paracentric inversions and a large reciprocal translocation between the two large chromosomes.

Two transplants of *D. hesperium pallescens* from among those of five localities were found to have the meiotic irregularities that are characteristic of structural heterozygosity. A bridge and fragment were found, for example, in 1 to 2 per cent of anaphase cells in one plant, indicating a paracentric inversion, and a second plant, from another population showed a high degree of delayed separations between one pair of the larger chromosomes. Several cells of the latter had a micronucleus at telophase which probably resulted from chromosome exclusion because of the delayed separation. This phenomenon has been observed in several species of *Delphinium* (Lewis *et al.*, 1951) and may indicate a difference in homology along the length of the paired chromosomes. Structural differences of this sort could arise from introgression and our studies of this species group show clearly that introgression occurs between *D. hesperium* and *D. variegatum* (Epling and Lewis, 1952). We shall refer to this later.

One would expect an appreciable reduction in fertility in the hybrid between *D. hesperium* and *D. recurvatum* from the segregation of the translocated chromosomes because they frequently form two independently segregating pairs, and when a ring is formed it often separates with adjacent chromosomes going to the same pole. The structural heterozygosity we have observed is probably only a part of the total because many other structural differences could be concealed by the low chiasma frequency, which is usually one per bivalent for the six short chromosomes and two per bivalent in the two long ones (Mehlquist, Blodgett, and Bruscia, 1943; Lewis *et al.*, 1951). This inference that much or perhaps all of the sterility of the *recurvatum-hesperium* cross is the result of structural differences is strengthened by comparison with an allotetraploid from this cross, which we induced by colchicine. When selfed, this allotetraploid produced about 10 seeds per follicle in contrast to an average of one seed in the diploid hybrid. Their germination (15 per cent) was comparable to that of the seeds of the selfed diploid hybrid. The allotetraploid was not as fertile as either parent but the reason was apparent from an examination of meiosis where it could be seen that the chromosomes frequently did not pair autosyndetically. Rings of 4 or chains of 3 or 4 involving the rearranged large chromosomes of the genome were frequent, and association as great as a ring of eight was observed. In addition, fertility may have been reduced by crossing over within heterozygous inversions because seven out of 135 first telophase cells examined showed a bridge, usually accompanied by a fragment.

Variation

Interspecific hybrids generally have a narrow range of variation in the first generation (Anderson, 1949). We have found, however, that the F_1's of *D. re-*

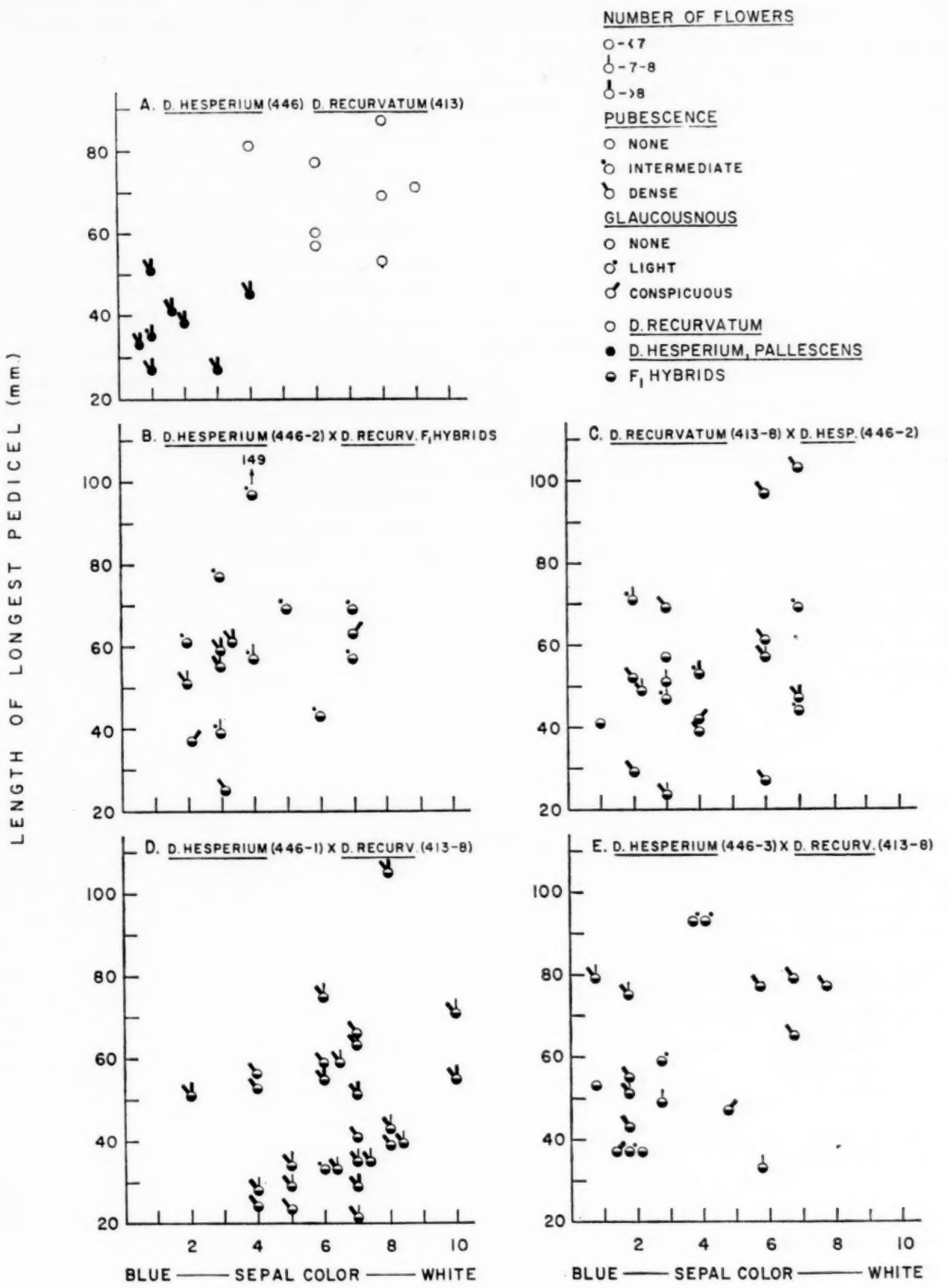


FIG. 3. Scatter diagrams showing the characteristics of certain hybrids between *D. hesperium pallescens* and *D. recurvatum*.

curvatum × *hesperium* are remarkably variable, approaching the variance to be expected in an F_2 progeny and exceeding by far that found in the progenies of intraspecific crosses. This variance is illustrated in figure 3. The abscissa of these scatter diagrams records the differences in sepal color from the white of

D. hesperium to the blue of *D. recurvatum,* following Maerz and Paul, and the ordinate records the length of the longest pedicel. Other characters are recorded by the whiskers on each ideograph, according to the legend: flower number, pubescence, and glaucousness. These are the more easily contrasted traits of these species. In the examples shown, one plant of *D. recurvatum* (413-8) was used in making crosses with three plants of *D. hesperium* from the same colony (446-1, 2, 3).

The range of variation of the F_1 hybrids of this cross clearly exceeds that of the parents. Inasmuch as the same plant of *D. recurvatum* was used in each case, the differences between crosses must have come from the different individuals of *D. hesperium,* and it seems likely that part of the concealed variability arose from previous introgression of another species, *D. variegatum,* with our parental population. *Delphinium hesperium* and *D. variegatum* are partly sympatric and are known to hybridize when in contact with each other. A clue to this introgression is found in the differences in pubescence graphed in figure 3. All of the individuals in one of the hybrid progenies had pubescent stems; whereas some individuals in the other three had glabrous stems. Since hybrids between *D. recurvatum* and *D. hesperium* characteristically have pubescent stems whereas hybrids between *D. recurvatum* and *D. variegatum* consistently have glabrous stems, the segregating F_1 progenies strongly suggest that the *D. hesperium* parents were introgressants with *D. variegatum* although such introgression was not evident from the morphology of the parents.

Among 374 F_1 *D. hesperium* × *D. recurvatum* plants examined, some were found that closely approached *D. gypso-*

TABLE 4. *Fertility of the F_1 hybrids indicated.* Female parent is listed first except in original crosses

	Number of plants pollinated	Number of carpels pollinated	Mean seed set per follicle	Seeds sown	Seeds germinated	Per cent germination
D. recurvatum × *D. hesperium**						
F_1 × self	125	1829	1.0	1710	178	10
× F_1	93	676	.7	371	78	21
× *recurvatum*	38	321	1.9	534	222	42
× *hesperium*	30	243	2.0	310	94	30
× *gypsophilum*	14	138	3.2	286	149	52
recurvatum × F_1	6	60	7.7	502	183	36
D. recurvatum × *D. gypsophilum***						
F_1 × self	27	261	19.7	2900	607	21
× F_1	25	225	23.1	1000	380	38
× *recurvatum*	14	126	19.8	628	270	43
× *gypsophilum*	4	36	23.5	570	250	44
D. gypsophilum × *D. hesperium****						
F_1 × self	2	27	1.5	32	2	6
× *hesperium*	1	9	6.0	50	30	60

* F_1's from 9 combinations including reciprocal crosses.
** F_1's from 6 combinations including reciprocal crosses.
*** F_1's from 1 cross.

philum in respect to particular traits such as leaf shape or flower conformation but differed conspicuously from that species in other traits. However, one individual (2196-1) from the cross *hesperium 445-4* × *recurvatum 413-8* was indistinguishable from *D. gypsophilum* even after a careful comparison. It proved to be a triploid with two genomes of *D. recurvatum* and one of *D. hesperium.* No progeny were obtained from it.

The F_2, backcross, and subsequent generations produced a vast array of variants, some recombining parental traits and others combinations unlike those of either parent. Some individuals were dwarfs, others commenced flowering in the rosette, some had 6-merous or otherwise abnormal flowers, or abnormal branching in the inflorescence. Some backcrosses were scarcely distinguishable from one parent or the other, and some crosses between *recurvatum*-like individuals produced progenies of which most if not all would have passed for this species in a wild population. When selfed, some individuals of such progenies would betray their hybrid origin, just as one might expect of naturally produced introgressants.

The hybrid derivatives of greatest interest to our study were 35 individuals that very closely resembled or were not separable from *D. gypsophilum* as it occurs over a wide geographic area. The progenies that produced these individuals are shown in table 5. Most were obtained by backcrossing to *recurvatum,* mostly 413. None was obtained from backcrossing to *D. hesperium.* The fertility of these *gypsophilum*-like plants, measured by seed set, was variable but was often as high as that of either parental species and in some instances as high as that of *D. gypsophilum.* The inference is reasonable that some of the hybrids recovered genomes structurally comparable to those of *D. recurvatum* after backcrossing to it. Progenies from *gypsophilum*-like individuals showed a limited amount of segregation around a *gypsophilum*-like mode, but it seems not unlikely that a sufficient number of trials would have produced a true breeding race, had we made them.

TABLE 5. *The derivation of individuals closely resembling* Delphinium gypsophilum *from hybrids between* D. recurvatum *and* D. hesperium pallescens

	Number of plants	Number closely resembling *D. gypsophilum*
Hybrids		
F_1	374	1*
F_2	238	0
$F_2 \times F_1$	36	0
F_3	128	5
F_4	506	0
$F_2 \times$ *recurvatum*	37	0
$F_2 \times$ *hesperium*	36	0
($F_2 \times$ *recurvatum*) × self	46	0
$F_3 \times (F_2 \times$ *recurvatum*)	16	0
Backcrosses to *D. recurvatum*		
B_1	417	3
$B_1 \times$ self	41	0
$B_1 \times B_1$	120	11
$B_1 \times F_2$	56	9
B_2	43	5
$B_2 \times$ self	331	1
B_3	205	0
Backcrosses to *D. hesperium*		
B_1	70	0
$B_1 \times$ self	146	0
$B_1 \times B_1$	27	0
B_2	230	0

* Triploid, see text.

DISCUSSION

From relatively few initial crosses between two strongly differentiated species, *Delphinium hesperium* and *D. recurvatum,* we have obtained a series of hybrid individuals that so strongly resemble a third wild species that they can be differentiated from it only after careful detailed comparisons or not at all. The parental species are not only morphologically distinct; they occupy quite different environments and are associated with plant communities which themselves have long and different histories. They have doubtless

been independent lines of evolution for a long period.

The species do not now grow within pollinating distance of each other, but because of the variable and changing landscape of the western cordillera during Cenozoic time, coupled with a fluctuating and changing climate, we find little reason to doubt that they may once have been in contact. Even today, being separated by only five miles at their closest point, the parental species are still doubtless within the range of seed transfer. We have no direct evidence that the seeds of either of these species will germinate within pollinating range of the other. We know from experiment, however, that this is possible with other species (Epling and Lewis, 1952). The terrain and habitats around most colonies are diverse and broken. One can surmise that a seed of one species might find lodgment in a tolerable habitat near a colony of the other even though the latter might occupy a very different environment. With a favorable season it might germinate and, given sufficient time and the protective trait of root dormancy, a series of hybrids might be formed and their seeds in turn might be transported to an environment in which natural selection might then develop a stable taxon. We can visualize the origin of *D. gypsophilum* in that way. No substantial reduction of fertility between it and its parents was required for its formation and the principal deterrents to present gene exchange are distance and habitat preference. Its formation was made possible, in fact, *because gene exchange between its parents was possible.*

We do not regard this process of rapid reticulate speciation as an isolated or unique event in *Delphinium.* The example described is one in which the process has reached a definitive stage and the derived species is a widespread integrated taxon. We are acquainted with what may have been its incipient stages by several examples of hybridization between other species that are similarly connectant (fig. 4).

D. parishii and *D. parryi,* for example, are two species as sharply defined as *D. hesperium* and *D. recurvatum.* The former chiefly inhabits communities found on the alluvial outwashes from the mountains that ring or are contained within the deserts of southern California and Arizona. The second is confined to the more arid aspects of the coastal vegetation of southern California, particularly the chaparral. The whole cycle of seasonal development is different in the communities in which each species finds its place. Because of variable topography and weather these communities frequently interdigitate and may produce fairly broad ecotones at some places. When they do so, *D. parishii* and *D. parryi* may be found in sufficiently close proximity to each other to exchange genes. We know of two areas, in Riverside and Ventura Counties, in which highly variable intermediate populations, or hybrid "swarms," indicate current or recent hybridization. In addition, we know of three areas in which uniform intermediate populations occur. The fact is of importance to our hypothesis that although these uniform populations are all derivatives of the same parental pair of species, they are measurably different from each other and recognizable. They represent three independent pools of interspecific recombination and resemble in different ways progeny we have obtained from hybridizing *D. parishii* and *D. parryi,* as well as individuals found in hybrid swarms elsewhere. Each occurs in an ecotone or marginal area, and their morphological uniformity shows that each has become fairly well stabilized. One is found in San Diego County in a small area along the Banner grade, east of Julian, where the chaparral is marginal to the Sonoran vegetation. This taxon has been formally described as *D. subglobosum* Wiggins. It resembles *D. parryi* more than *D. parishii.* It is a single population as far as we know. The second has a more extensive range at low elevations along the western margin of the Colorado desert in San Diego

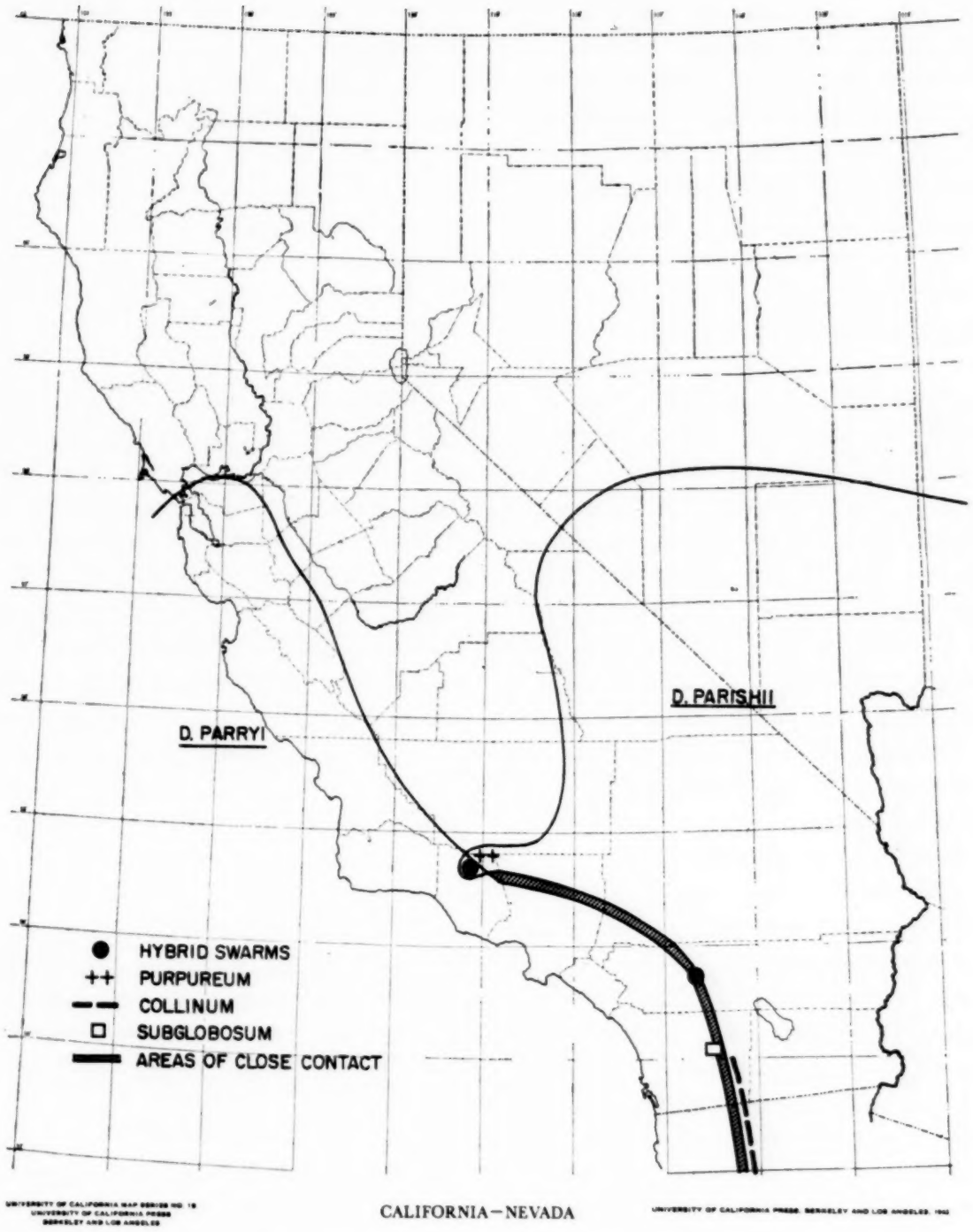


FIG. 4. Approximate geographic ranges of *D. parryi* and *D. parishii*, together with the positions of the hybrid swarms and stabilized taxa referred to in the text.

County and adjacent Baja California. It has been described as *D. collinum* Ewan. It resembles *D. parishii* more than *D. parryi*, and occurs in a habitat which more closely resembles that of *D. parishii*. It grows associated with desert shrubs at the base of the mountains, particularly near the mouths of canyons, where large colonies are found intermittently for a distance of more than fifty miles. The third occurs in Cuddy and Lockwood Valleys in southern Kern and northern Ventura counties. Minor differences distinguish the colonies in Cuddy Valley from those in Lockwood Valley but both are morphologically more similar to *D. parishii* than to *D. parryi*. In both areas they are growing with sagebrush (*Artemisia tridentata sensu lato*) adjacent to pinon-juniper woodland.

For taxonomic convenience we have grouped these three stabilized intermediate populations under *D. parishii*. Those from San Diego County (*D. subglobosum* and *D. collinum*) we have referred to subsp. *subglobosum* and those from Kern

and Ventura counties to subsp. *purpureum* (Lewis and Epling, 1954).

We believe that these hybrid swarms and stabilized intermediate populations between *D. parryi* and *D. parishii* may very well illustrate stages that occurred in the evolution of *D. gypsophilum*. The colony of *D. subglobosum* only rarely comes into flower and frequently does not even appear as rosettes. We were fortunate one year in getting a considerable series of transplants. They proved to be remarkably uniform. But the colony is very restricted and may not occur elsewhere. With expected changes in topography and climate, it may be extinguished; or should these changes (and the new plant associations that would follow) extend its habitat, it might then increase its range. *D. collinum* has apparently done this and, as we believe, *D. gypsophilum* also. The habitat of the latter is assuredly intermediate between those of the oak woodland and the saline playas in which its parents grow.

Few will doubt that the process we describe is one of evolution. New, well defined, and adaptively different taxa have been formed by hybridization without change of chromosome number and have multiplied and spread in newly evolving habitats. One might regard this process as a component of phyletic evolution (Simpson, 1944) because *Delphinium* and the closely related genus *Aconitum* represent a very ancient, distinct, and morphologically circumscribed phylad that has not greatly transcended certain environments of the northern hemisphere. So far as we can judge from crosses made by Professor G. A. L. Mehlquist and by us, interspecific fertility is commonly present in *Delphinium*, giving thus the possibility of repeatedly producing new taxa in nature of the sort we have described. These data, combined with our observations of natural populations, show clearly that interspecific sterility has not developed between many well defined and ancient taxa. The fact that interspecific hybridization can result in the formation of new species by amphiploidy is no longer novel. The importance of our data is to indicate that genetic recombination and a new synthesis may also come rapidly at the diploid level. The rapidity of the adaptive spread of a population of hybrid origin and its establishment and genetic integration as a species would depend on the then prevailing rates of environmental change, which might be either local or general.

Opinion may be less than unanimous that this process results in speciation, inasmuch as final barriers seem seldom to have arisen in *Delphinium*. By this criterion, *Delphinium* would appear to be a long continued anastomosing phylad within which species are approached but seldom realized. Nevertheless, well integrated and independent lines of evolution are formed that may persist for long periods without distintegration even though they may frequently be in contact and interfertile. We regard them as species.

We believe, therefore, that *Delphinium gypsophilum* has good claim to specific rank in spite of its interfertility with other members of its alliance, and that it was formed by interspecific hybridization without change of chromosome number, in the manner described.

Summary

Delphinium gypsophilum is a well defined, wide spread species found at lower elevations in the foothills of the coastal and cross ranges that border the central valley of California. It is morphologically intermediate between two other widespread species and occupies a habitat that is geographically and ecologically intermediate. Evidence is discussed that suggests that *D. gypsophilum* had its origin by a rapid sequence of hybridizations and fixations of a limited number of hybrid genotypes, without change of chromosome number. Other evidence indicates that the process of reticulate anastomosing speciation represented by this example is a general attribute of *Delphinium*, because the barriers to interspe-

cific gene exchanges are seldom absolute and are commonly moderate. Our criterion for speciation is the persistence for indefinite periods of well defined, recognizably stabilized taxa, even though they may frequently be in sufficient contact to exchange genes abundantly.

Literature Cited

Anderson, E. 1949. Introgressive Hybridization. New York. Wiley & Sons, Inc.

Epling, C. 1947. Actual and potential gene flow in natural populations. Am. Nat., **81**: 104–113.

—— and H. Lewis. 1952. Increase of the adaptive range of the genus *Delphinium*. Evolution, **6**: 253–267.

Lewis, H. 1947. Leaf variation in *Delphinium variegatum*. Bull. Torrey Bot. Club, **74**: 57–59.

—— and C. Epling. 1946. Formation of a diploid species of *Delphinium* by hybridization. (Abstract). Amer. Jour. Bot., **33**: 21s–22s.

—— and ——. 1954. A taxonomic study of Californian delphiniums. Brittonia, **8**: 1–22.

——, ——, G. A. L. Mehlquist and C. G. Wyckoff. 1951. Chromosome numbers in California Delphiniums and their geographical occurrence. Annals Mo. Bot. Gard., **38**: 101–118.

Maerz, A., and M. R. Paul. 1930. A Dictionary of Color. New York. McGraw Hill. 1st Edition.

Mehlquist, G. A. L., O. Blodgett and L. Brascia. 1943. Colchicine induced tetraploidy in *Delphinium cardinale*. Jour. Hered., **34**: 187–192.

Simpson, G. G. 1944. Tempo and Mode in Evolution. New York. Columbia University Press.

Stebbins, G. L., Jr. 1950. Variation and Evolution in Plants. New York. Columbia University Press.

CHROMOSOMAL DISTRIBUTION OF MUTATOR- AND RADIATION-INDUCED MUTATIONS IN *D. MELANOGASTER*[1]

P. T. Ives

Department of Biology, Amherst College, Amherst, Massachusetts

Received April 13, 1959

Studies with the mutator *hi* (Ives, 1950; Hinton, Ives and Evans, 1952) and with low and high doses of Cobalt-60 γ radiation (Ives, 1958, 1959) produced three extensive series of sex-linked lethal mutations which were analyzed genetically for association with gross chromosomal aberrations. That analysis also provided information on the distribution of "point mutation" lethal loci along the X-chromosome in each of the three series of mutations. It is that phase of the work which will be presented and discussed in this report.

While considerable information on the distribution of radiation induced mutants in particular has been published in the last thirty years (see Muller's, 1954, review) the lethals from the author's experiments constitute perhaps the most extensive series analyzed genetically in one laboratory. They are of particular interest because they provide a chance for comparison between not only what are essentially spontaneous lethals on the one hand and radiation ones on the other, but also between lethals induced by a much lower dose (300 r) than is usually employed and those induced by the highest dose (12.5 kr) which has yet been used extensively in mutagenic work with *Drosophila*. It seems worthwhile, therefore, to record the chromosomal distribution of these lethals even though the analysis must be limited for the most part to large chromosome areas, because of the technical limitations of the study.

[1] This work was done under Contract AT (30-1)-930 with the United States Atomic Energy Commission.

Methods

All of the mutations which are included in this distribution analysis were tested for chromosome locus by use of the marker stock, *y ct*^{6} *ras*^{2} *f* (Bridges and Brehme, 1944). This stock proved to be technically the best of several which were tried in the early stages of work with *hi* mutations. In general, mutants have been included in the distribution analysis only when their crossover counts were large enough to produce a minimum of ten crossovers in each of the three regions enclosed by the marker genes, and only when a normal amount of crossing over was indicated in each of those regions. Crossing over was considered abnormal when recovered crossovers in any one of the three regions totaled less than half the number expected on the basis of its genetic map length. In cases where crossing over appeared to be low retests were made, and females were also classed for crossovers so as to detect cases in which two lethal loci occurred together in one lethal chromosome.

Only lethal mutations were included in the distribution analyses of the radiation series, the lethal distinction being based chiefly on the count in one test generation (Ives, 1959). During the work with *hi*, final classification of a mutant as lethal depended generally upon the results of several generations of counts. During that time some lines which would have been lethal on the basis used in the radiation tests became semi-lethal and usually showed some abnormal phenotype in addition. Because all counts for any one mutant were added together for the rec-

ord now at hand it is not possible to regroup the lethals in the mutator series on the same first-count basis as was used in the radiation series. As it turns out, however, the distribution of mutator visibles along the X-chromosome is not statistically different from that of the lethals so that it makes little difference which grouping of *hi* mutants one uses in the distribution comparison, lethals alone or lethals and visibles together.

It seems best not to base the distribution comparison of the mutants in these three series primarily on the use of arbitrary short regions of the X-chromosome, five or ten crossover units in length. In the radiation series most crossover counts totaled only between 100 and 150 test males, a number small enough to produce too much random variation for the use of such short chromosome regions. Tests of the mutator lethals showed a wide variation in size of crossover count, some of them as high as several hundred males. In addition, those tests were carried out several years earlier on a different food formula and with the aid of a different assistant in scoring crossovers. While the three variables, size of count, food, and scorer, should not affect the location of the mutator lethals with respect to the marker genes, they might well have a significant effect on the estimate of their specific locus within any one of the four regions set off by the marker genes. Accordingly no attempt has been made to localize the *hi* mutations beyond placing them in one or another of the regions defined by the markers. The radiation lethals were tested under more uniform conditions, and are, therefore, also grouped by five-unit regions, for whatever such grouping may be worth.

Lethals which proved to be phenotypically allelic to any one of the marker genes, presumably because of a deficiency or a deletion, are not included in the distribution analysis. No such cases occurred in the mutator tests but several appeared in each radiation series.

In addition to comparing the distributions between series of lethals it is also of interest to compare each distribution to the amount of genetic material available within each chromosome region. Probably the best estimate for this purpose is the proportion of total bands of the salivary gland X-chromosome which are included in each of the four regions, using the Bridges (1938) map.

Presentation of Data

The distribution data are given in tables 1, 2 and 3. In table 1 are the numbers of lethals in each series which have their loci in each of the four numbered regions set off by the marker genes and the numbers of lethals which were too closely linked to the marker loci to separate from them by crossing over in the comparatively small number of crossover counts which were scored. It can be seen that crowding is especially prominent in the region near *y* where crossing over is normally much lower than in the areas occupied by the other marker genes.

For the grouping in table 2 the lethals not separated from y^+ by crossing over were all considered as lying between that locus and the ct^+ locus, designated in the table as the *y-ct* region. Those lethals similarly close to the other markers were divided evenly between the two regions adjacent to the marker in each case, the "odd" member in any such group being distributed at random to one region or

Table 1. *Distribution of Lethals in the X-chromosome*

Series	Total	Region							
		y	1	*ct*	2	*ras*	3	*f*	4
Mutator	250	20	31	4	62	9	86	14	24
300 r	324	37	62	5	44	4	110	10	52
12.5 kr	176	17	35	3	24	4	46	11	36

TABLE 2. *Percentage Distribution of X-Mutations by Regions*

Series	Total	*y–ct*	%	*ct–ras*	%	*ras–f*	%	*f–end*	%
1. Mutator	250	53	21	69	28	97	39	31	12
2. Mut. vis.	42	14	33	6	14	17	41	5	12
3. 1 + 2	292	67	23	75	26	114	39	36	12
4. 300 r	324	101	31	49	15	117	36	57	18
5. 12.5 kr	176	53	30	28	16	54	31	41	23
6. 4 + 5	500	154	31	77	15	171	34	98	20
7. Sal. Bands			32		10		32		26

the other. Thus all of the lethal mutations of table 1 were placed in the four regions indicated in table 2 and percentages of each series lying in each region were calculated.

Item 2 in table 2 shows the distribution of the 42 visible mutations in the mutator series referred to above. The value of P for the goodness of fit of the two series of mutator mutants, items 1 and 2 in table 2, is approximately .21 by chi square, indicating that the two distributions do not differ significantly from each other. Similarly, the distribution of the lethal loci in the two radiation series, items 4 and 5 resemble each other closely, with a P value of .35 for their comparison.

The mutator lethals, and the combined data for mutator lethals and visibles together, show distributions quite different from that observed in either radiation series, significant at lower than the 1% level in any one of the major comparisons —items 1 vs. 4, 1 vs. 5, 1 vs. 6, 3 vs. 5, 3 vs. 6 in table 2. The mutator visibles are too few in number to warrant separate comparison with the radiation series. As it happens their distribution is much more like the distribution of radiation lethals than that of the mutator lethals. Their addition to the mutator lethals in item 3 does not alter the general character of the distribution compared to that of lethals alone in item 1.

Comparison of the mutation distributions with the proportionate distribution of salivary gland X-chromosome bands, item 7 of table 2, shows at a glance that the mutator mutants in items 1 and 3 differ much more than the radiation mutants do in this comparison. There is obviously a surplus of mutator lethals in the *ct-ras* region. In addition to that, the comparative proportions of mutator lethals in the other three regions do not resemble the proportions in item 7. The proportions in the radiation series, separately and together, seem to the author to be remarkably like the proportions in the "salivary bands." Because of the roughness of these estimates and the uncertain numerical relationship between

TABLE 3. *Distribution of Radiation Lethals in Five-Unit Regions*

	Region														
Series	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total
300 r	60	12	16	11	23	22	10	25	23	25	24	34	20	19	324
12.5 kr	23	6	15	8	13	10	11	8	8	17	9	26	14	8	176
Both	83 (y^+)	18	31	19	36 (ct^+)	32	21 (ras^+)	33	31	42	33	60 (f^+)	34	27	500

bands and genes the author has not calculated a P value for the comparison of item 7 to the other items in table 2.

Table 3 shows the distribution of radiation lethals in the successive five-unit map regions of the X-chromosome, from 0.0–4.9, 5.0–9.9, etc., to region 14 which includes all lethals beyond 64.9, the genetic map itself ending at 66.0 units. As indicated, the marker genes fall in regions 1, 5, 7, and 12. The y^+ and f^+ areas are the two most heavily populated regions in both series. The crowding at the y^+ end has already been discussed.

The concentration of lethals around f^+, which is also apparent for mutator lethals in table 1, may be due to either of two causes. First, there may actually be a group of genes with exceptional mutational characteristics in this area, such as seems to be the case for certain visibles in the *ss-e* region in the third chromosome (Ives, in press). Secondly, this might be due in part to a certain possible class of small inversions beyond f^+, or perhaps translocations with their X-break close to the kinetechore, which could have the property of sharply reducing crossing over in the immediate vicinity of *f* and out to the kinetechore without notably reducing crossing over in the *ras-f* region as a whole.

In this second condition one would expect a larger proportion of such lethals in region 12 in the 12.5 kr series than in the 300 r series since there was an eight-fold difference in apparent gross chromosomal aberrations in the two series (Ives, 1959). The actual difference is only about two-fold, assuming the occurrence of an average proportion of point lethals in region 12 in each series. One would not expect this phenomenon to affect the proportions of lethals found in regions 13 and 14 in the two series. The data in table 3 are in accord with that expectation.

The crossover map gives to region 14 only one-fifth of the area represented in the other regions. As it happens some of the lethals in this region seemed to have loci well beyond the end of the map (66.0), out in the 70's. These could be cases where the lethal became semi-lethal when operating in the different genetic background brought in by the $y\ ct^6\ ras^2\ f$ stock. Or they may represent variation arising from small counts, combined with the viability advantage of the wild-type single crossover flies. In any case, there are more lethals in region 14 than expected solely on the proportion of the standard map represented in that region.

It is doubtful if a chi-square analysis of the regional distributions in table 3 can add substantially to the information gained by sight analysis, because of the double scattering effect of random lethal distribution and small crossover counts. On the basis of such an analysis, the frequencies of lethals region by region do not differ significantly between doses (P is .42), and the distribution within regions other than 1 and 12 is not strikingly different from random (P is .06). At least this is in line with the more significant comparisons of the distributions in table 2.

Discussion

The results of the distribution analysis of series of sex-linked lethals produced on the one hand by a mutator and on the other by ionizing radiation indicate that while low and high doses of γ radiation give similar chromosomal distributions of lethal loci, which are also similar to the distribution of available genes, the mutator lethals show a distribution which is quite different from the other distributions.

This difference in distribution presumably reflects a general difference between the mutator effects and radiation effects. The possibility of influence of some other genetic differences besides the mutator cannot be entirely ruled out in the present case. This possibility arises in part because of the several years time difference between the tests with *hi* and those with γ radiation. In addition, the stock used in the radiation study was a long-inbred line, derived may years previously from

Roseburg, Oregon, while the stock used in the *hi* tests was a mixture of chromosomes from stocks with a long history of relatively close-breeding in the laboratory (one of them known to originate in Michigan) and of chromosomes derived a few years previously from Winter Park, Florida. The actual X-chromosomes used in both studies had been cultured in the laboratory for more than twenty years before these studies began with the result that they were probably genetically quite different from their original conditions and are more alike than their Florida-Michigan-Oregon origins suggest.

The mutagenic effect of a genetic mutator such as *hi* is presumably much more specific biochemically than are the mutagenic effects of ionizing radiation such as Cobalt-60 γ rays. It can be hypothesized, therefore, that *hi,* even though it has been shown to affect many loci, will not affect them either as equally or as randomly as they are affected by γ radiation, mutagenically. On this basis one would expect (a) that the distribution of lethal loci along the X-chromosome will be different in series of lethals produced on the one hand by a genetic mutator and on the other hand by ionizing radiation, and (b) that the chromosomal distribution of lethal loci in the radiation series will more nearly approximate the distribution of available genetic material. Both of these expectations are borne out in the present data. (These expectations cannot be labeled predictions because this argument was not conceived, by the author at least, until after the data were grouped in tables 1 and 2 of this report.)

The earlier data on visible mutants (Ives, 1950) suggested that different mutators affect various loci differentially. In view of this fact and the present findings with respect to distribution of sex-linked lethal loci, it is reasonable to predict that different mutators will also produce different distributions of lethal loci in the X-chromosome. If most spontaneous mutations are produced by mutators, by the "lowest rate" alleles which can result from the selection process at a given time, then it may also be predicted that series of lethals occurring spontaneously in stocks of different recent geographical origin will sometimes show distinctly different chromosomal distributions. But sex-linked lethals induced by ionizing radiation in the same stocks should be at least less different in their distribution than the spontaneous series, providing they are induced under otherwise similar experimental conditions.

It would be a difficult task, probably involving a great deal of just plain luck, to produce from other geographic strains mutator data comparable quantitatively to that which resulted from the work with *hi,* a mutator that ceased to be useful for experimental work several years ago. It would not involve such fortuitous circumstances to study the distribution of radiation induced lethals in stocks of different recent geographical origin. Such a task is also made easier by the close similarity of the 300 r and 12.5 kr series of lethals in the present study. It appears that one can use a more convenient dose than either of these extremes and reasonably expect results which are definitive for the whole range of possible doses.

One is tempted to cite the similarity of the 300 r and 12.5 kr distributions in the present study as favoring the view that these lethals, whatever their nature, are independent of gross chromosomal aberrations, since the latter appear to be several times more numerous in the 12.5 kr tests than in the 300 r tests. However, as pointed out in considering the difference in rate-dose relationship in sex-linked lethals vs. dominant visibles (Ives, in press), such a distinction cannot be made on the basis of such data as these. It is not unlikely that lethal chromosome breaks which lead to or are caused by induced chromosomal aberrations are also distributed randomly to the same extent as point mutations, the two distributions as similar perhaps as those of the 300 r and 12.5 kr series recorded here. If so,

then removing lethals which are associated with gross chromosomal rearrangements, either by genetic tests as was done here, or by loss of cell lineages as is known to happen during meiosis of diploid cells heterozygous for gross chromosomal rearrangements, should leave a residue of lethals, whether restitution lethals or point mutation lethals, whose distribution is unchanged from that which existed for all lethals together before any elimination took place.

Summary

1. Three series of X-chromosome lethal mutations, from the mutator *hi,* from 300 r and from 12.5 kr of cobalt-60 γ radiation, all showing normal crossing over with *y* ct^6 ras^2 *f,* were analyzed for distribution of lethal loci, chiefly with respect to the four regions set off by the marker genes.

2. The distributions are compared to each other and to proportions of available genetic material (as indicated by salivary gland chromosome bands) in each region.

3. The radiation series show similar distributions and are also similar to the proportionate distribution of bands. The mutator series differs strongly from the others, with an especially high concentration of lethals in the *ct-ras* region and different relative proportions of lethals in the other regions.

4. There is an apparent and moderate concentration of lethal loci near f^+ in each of the three series. This may reflect either a group of genes with exceptional mutagenic properties or the occurrence of chromosome aberrations between *f* and the kinetechore which do not reduce crossing over sufficiently in the *ras-f* region to be detected by the methods used here.

5. The difference between mutator and radiation lethal loci distribution is consistent with the hypothesis that mutator genes are genetically more specific, ionizing radiations more general, in their mutagenic effects.

6. It is suggested that series of "spontaneous" lethals from strains of *D. melanogaster* derived recently from different geographic areas may be expected sometimes to show different chromosomal distributions but that series of lethals induced in such strains by a given radiation treatment should be generally alike.

7. These data are not critical for a distinction between the various hypotheses concerning the genetic nature of lethal mutations.

Acknowledgments

The author gratefully acknowledges the invaluable assistance of Mrs. Barbara Scott, Mrs. Virginia Reed White and especially Mrs. Lucy Casey in the laborious crossover tests. He is also grateful to Professor W. M. Hexter for criticizing the manuscript.

Literature Cited

Bridges, C. B. 1938. A revised map of the salivary gland X-chromosome of Drosophila melanogaster. J. Hered., **29:** 11–13.

Bridges, C. B., and K. S. Brehme. 1944. The mutants of Drosophila melanogaster. Carnegie Inst. Wash. Pub. **552.** vii + 257 pp.

Hinton, T., P. T. Ives and Alice T. Evans. 1952. Changing the gene order and number in natural populations. Evolution, **6:** 19–28.

Ives, P. T. 1950. The importance of mutation rate genes in evolution. Evolution, **4:** 236–252.

——. 1958. The mutation rate in Drosophila sperm after cobalt-60 γ radiation. (Abst.) X Internat. Cong. Genetics, **2:** 132.

——. 1959. The mutation rate in Drosophila after high doses of gamma radiation. Proc. Nat. Acad. Sci., **45:** 188–192.

——. (in press) The relation between radiation dose and dominant visible mutation rate in Drosophila melanogaster. Genetics.

Muller. 1954. The nature of the genetic effects produced by radiation. Radiation Biology, vol. I: High Energy Radiation, Chap. 7, pp. 351–474. Ed. A. Hollaender. McGraw-Hill, N. Y.

ECOLOGICAL AND PHYSIOLOGICAL OBSERVATIONS ON POLYMORPHIC POPULATIONS OF THE TOAD *BUFO ARENARUM* HENSEL, FROM ARGENTINE

J. M. Cei

Instituto de Biologia, Universidad Nacional de Cuyo, Mendoza, Argentine, and Centro de Investigaciones Zoologicas, Universidad de Chile, Santiago de Chile

Received February 4, 1959

The distribution area of *Bufo arenarum* Hensel extends from southern Brazil southward to southern Patagonia. This toad species appears to be an ecologically very versatile form, since it occurs in a great variety of ecological niches available in its distribution regions, except for the high Cordilleras. Despite the absence of obvious natural barriers between the different populations, the latter often differ in a variety of characters, such as colors and patterns, certain reflexes, and metabolic rates. These differences seem to be correlated with environmental conditions. Particularly interesting are the differences observed between the populations of the arid deserts near Mendoza and those of San Luiz, Cordoba, and Tucuman. The following account may be regarded as an exploratory study which is needed for a more thorough analysis of the population structure of the species.

Materials and Methods

The specimens were collected in the following regions: (1) Mendoza (646 specimens of both sexes), Parque San Martin, Lujan-Maipu 20 kms eastward, Las Heras 10 kms westward; all these collecting sites are at elevations 700–800 m, in artificial ponds and pools. (2) San Luiz (70 specimens), El Volcan, elevation 900–1000 m, in little streams among hills. (3) Cordoba (38 specimens), Rio Cuarto, Rio Ajis, Alta Gracia, mostly breeding toads. (4) Tucuman (401 specimens), near the town of San Miguel, elevation 400–500 m, Rio Sali, some breeding specimens collected. (5) Buenos Aires (55 specimens), Avellaneda, near the town, not breeding individuals. Finally, some comparative data can be reported on the populations of *Bufo spinulosus* Wiegmann, a relative of *B. arenarum* living in Chile.

Cei (1956) has reported that some individuals in certain populations of *B. arenarum* exhibit the so-called hypnotic reflex, while other individuals do not. This behavioral trait may be seen in the photographs in plate 1. The reflex is easily provoked by a moderate pressure on the throat. The period of cataleptic inhibition is variable from individual to individual; it may last for 15 minutes and even longer. Life in captivity does not alter the reflex in those individuals who have it. The reflex may also be provoked in individuals suddenly plunged in water or pinched. It exists equally in breeding and in non-breeding individuals.

The characters of pigmentation have been scored in living specimens. Measurements on the metabolic rates (oxygen consumption) were made in glass chambers, with the classical volumetric technique. Water regulation was studied measuring the percentage of weight loss, during a controlled time, in standard glass chambers with $CaCl_2$ at temperatures between 23° and 26° C. Data on resistance of different populations to climatic changes were obtained using terraria of standard construction and capacity, the animals being kept under identical

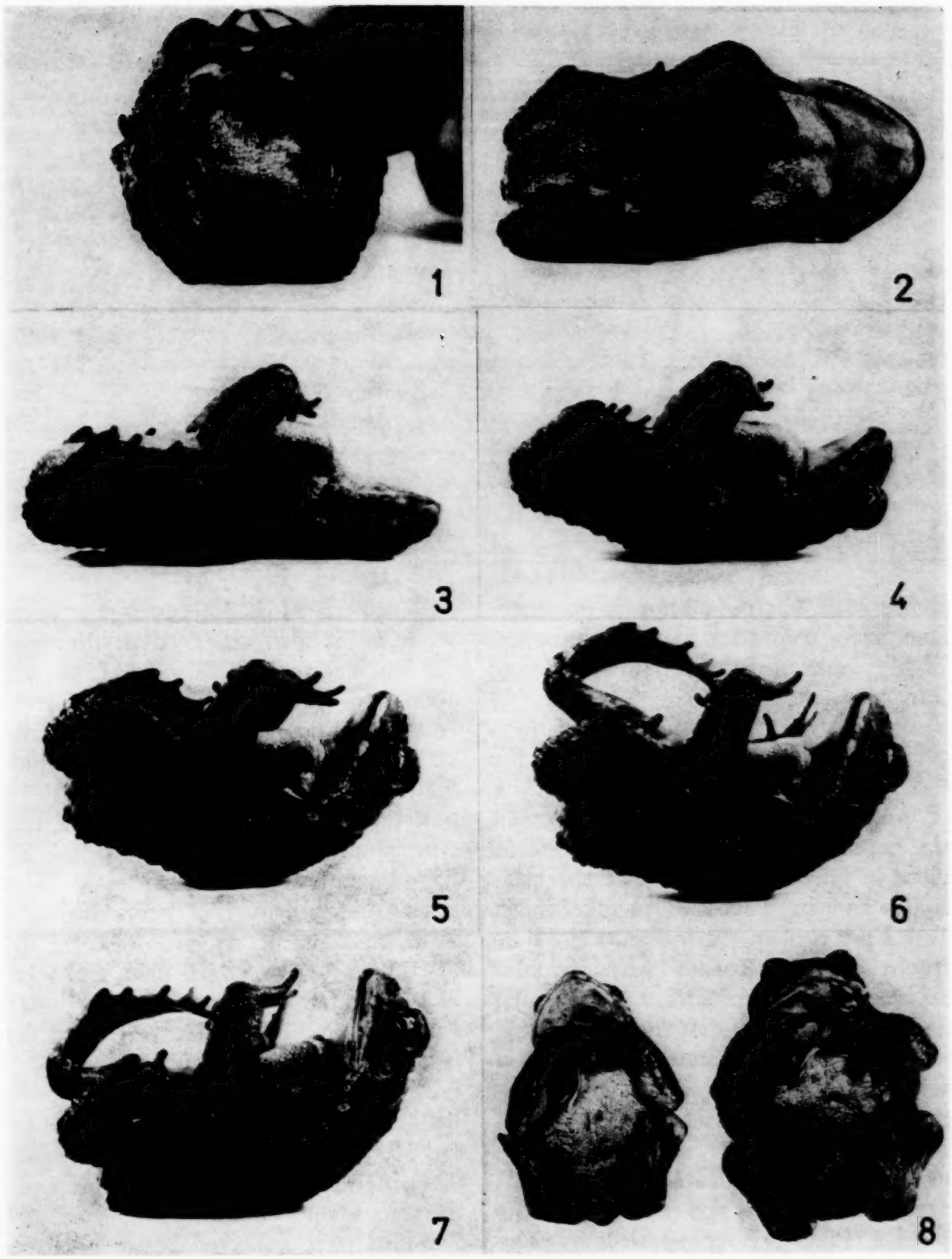


PLATE 1. The hypnotic reflex in the Mendoza, Argentine, population of *Bufo arenarum*. Nos. 1–3, manifestation of the reflex; Nos. 4–7, progressive loss of the inhibition. No. 8, the same reflex in a male of *Bufo spinulosus* from the population of Vallenar, Chile.

environmental conditions. All the observations on ecological resistance, water loss, and metabolic rates were made at Santiago de Chile, in the laboratory of the C. I. Z. (Centro Investigaciones Zoologicas de la Universidad de Chile) between December of 1956 and November of 1957.

THE OBSERVATIONS

The data on the coloration and on the hypnotic reflex are summarized in table

TABLE 1. *Frequencies of spotted and unspotted individuals, and of those showing and not showing the hypnotic reflex in different populations*

Localities	No yellow spots Reflex present	No yellow spots Reflex absent	Yellow spots Reflex present	Yellow spots Reflex absent
1. *Mendoza*				
San Martin	93	45	28	19
Las Heras	141	118	50	40
Lujan-Maipu	58	28	20	6
Total	292	181	98	65
2. *San Luiz*				
El Vulcan	17	53	—	—
3. *Cordoba*				
Rio Cuarto, Rio Ajis	—	27	—	—
Alta Gracia	—	11	—	—
4. *Tucuman*				
San Miguel, Rio Sali	7	393	—	1 (?)
5. *Buenos Aires*				
Avellaneda	—	55	—	—

1. The Mendoza population is polymorphic both for coloration and for the reflex, 25.2 per cent of the individuals having yellow spots, and 60.5 per cent showing the reflex. In the other populations spotted individuals are absent (except for one specimen with a very small yellow spot found at Tucuman). In the San Luiz population the hypnotic reflex is elicited in only 24.3 per cent of the individuals, and in Tucuman population in only 1.7 per cent of the individuals. The Cordoba and the Buenos Aires populations are monomorphic for both traits, i.e., unspotted and showing no reflex. The reflex and the yellow spots were also absent in the small sample of six specimens from Jorba (San Luiz), in several hundred of specimens inspected in the laboratory of the Institute of Biology M. Ferreira in Cordoba, and in 11 specimens from La Plata (Buenos Aires). The reflex and the yellow spots were both present in 42 specimens from San Carlos (120 km south of Mendoza) which is a very arid locality, and in five specimens from Potrerillo (foothills of the Andes facing Mendoza).

It appears, then, that the hypnotic reflex and the yellow spots occur in the populations of the arid regions of western Argentine. Most interesting, a parallel variation is also observed in the related Chilean species, *B. spinulosus,* of which about 300 individuals have been studied. In the arid desert of Atacama (Vallenar) 22.9 per cent of the individuals exhibited the hypnotic reflex, and 66 per cent of the individuals from the arid valley of Curacavi, south of Valparaiso. In the Santiago population the frequency of the reflex declines to 17.2 per cent, in the little streams of the coast of Paposa (Antofagasta) to 5.2 per cent, and to zero in the mountain streams of the Puna de Atacama and in the Central Cordilleras facing Santiago.

A simple experiment was carried out with the toads from the Cordoba and from the Mendoza populations, to demonstrate the different resistance of these populations to severe environmental conditions. Samples were collected between November 13th and December 5th, 1956, in Cordoba (Rio Ajis) and at Mendoza (Parque San Martin). The toads were taken to Santiago de Chile, and on December 5th placed in identical terraria, being cages with wire screens, and about 20 cm. deep sand on the bottom. The terraria were not protected against evaporation, which is strong in the summer

TABLE 2. *Mean weight (in gr.) and mean water loss (in per cent) in samples of ten individuals from Mendoza and ten individuals from Cordoba populations*

	Mendoza		Cordoba	
	Mean	Range	Mean	Range
Weight				
Initial	46.55 ± 3.87	30.6–66.9	59.34 ± 4.24	41.3–90.1
After 24 hours	42.59 ± 3.70	27.3–62.3	55.24 ± 4.00	38.4–83.4
After 48 hours	39.51 ± 3.75	24.3–59.8	51.80 ± 3.91	35.6–79.3
Water loss				
24 hours	8.88 ± 0.65	6.6–13.2	6.78 ± 0.26	5.4– 7.6
48 hours	16.26 ± 1.33	10.7–25.0	12.83 ± 0.31	11.1–14.4

climate of Santiago. The food was very scarce. Now, the sample of 24 male specimens from Cordoba, all without yellow spots and without the hypnotic reflex, showed a 50 per cent mortality after three months of captivity (by March 5th, 1957), and 92 per cent mortality after five and a half months (by May 21st, 1957). By June 5th all the specimens were dead. The sample of 12 male specimens from Mendoza, all with the hypnotic reflex and two specimens with yellow spots, showed no mortality after three months, 16 per cent mortality after five and a half months, 25 per cent after nine months and the same after 11 months when the experiment was discontinued. The difference between the mortality rates in the Mendoza and the Cordoba samples is very significant.

To study the tolerance of the Mendoza and Cordoba toads to water loss, the animals were placed between March 11th and 16th, 1957, in glass chambers in which the humidity was reduced by $CaCl_2$ (temperature 23°–26° C.). The results are shown in table 2. It can be seen that the Mendoza toads lose water even more rapidly than do the Cordoba toads (the probability of the difference observed being due to chance lies between 0.01 and 0.02 for the 24-hours experiment and between 0.02 and 0.05 for the 48-hours experiment). It should be noted in this connection that the Mendoza toads are smaller than those from Cordoba, and that the per cent of the water loss is, as shown in figures 9 and 10, inversely correlated with the body size. Large specimens lose water more slowly than do smaller ones. The correlation coefficients (r) computed for the data shown in nos. 2–5 of plate 1 are as follows:

	Mendoza	Cordoba
24 hours	−0.77	+0.45
48 hours	−0.75	−0.49

Examination of figures 1 and 2 also shows that the regression lines describing the relationships between body weight and per cent of water loss are steeper in

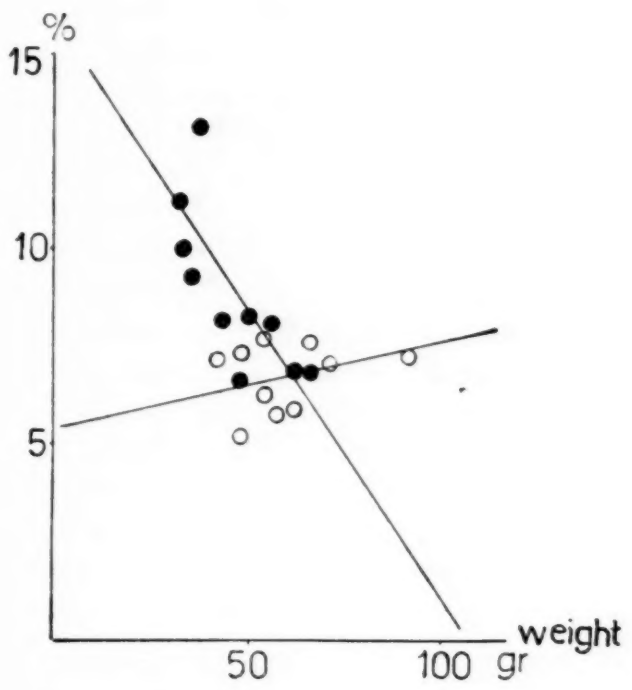


FIG. 1. Regression lines of the percent of water loss on the initial body weight (gr) in the Mendoza (filled circles) and Cordoba (empty circles) populations of *Bufo arenarum*, after a 24-hours-long exposure to desiccation.

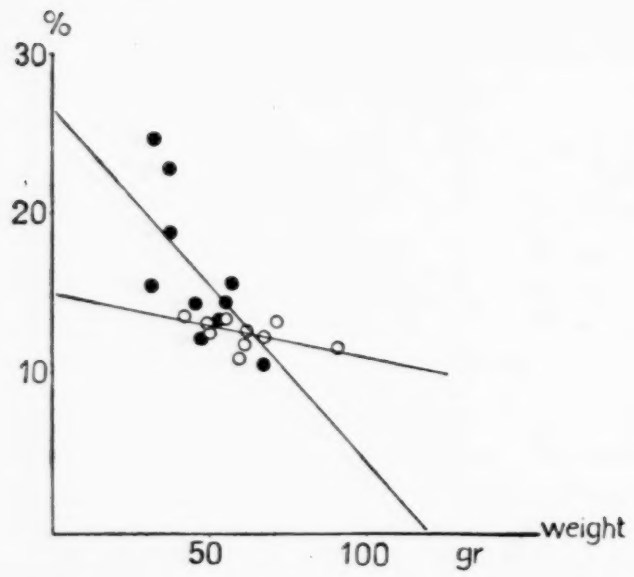


FIG. 2. Regression lines of the percent of water loss on the initial body weight (gr) in the Mendoza (filled circles) and Cordoba (empty circles) populations of *Bufo arenarum*, after a 48-hour-long exposure to desiccation.

the toads from Mendoza than in those from Cordoba. In fact, the former are strikingly similar to those obtained by Kirk and Hogben (1946) for *Bufo bufo* from England. The environmental conditions of Mendoza are, of course, completely unlike those in England.

SUMMARY

The populations of *Bufo arenarum* from Mendoza are polymorphic for a coloration character (presence and absence of yellow spots) and for a neurological character (presence or absence of the hypnotic reflex). The populations of Cordoba and of Buenos Aires are, as far as known, monomorphic, being unspotted and devoid of the hypnotic reflex. In Tucuman and San Luiz populations the coloration is monomorphic (unspotted), but both individuals with and without the hypnotic reflex are found.

It is surmised that the polymorphism is adaptive in the arid environments of western Argentine. Experimental tests do show that the toads from Mendoza survive longer under stringent conditions of dryness and starvation than do the toads from Cordoba. Nevertheless, direct measurements showed a greater rate of water loss and a higher metabolic rate in the Mendoza than in the Cordoba toads. Further observations are needed to unravel the physiological mechanisms which contribute to the higher adaptedness exhibited by the Mendoza populations under the conditions of the experiments.

ACKNOWLEDGMENTS

The author wishes to thank the C. I. Z. (Centro Investigaciones Zoologicas) of the University of Chile (Director, Prof. G. Mann) for the help received during the work. He is obligated also to Dr. J. Concha, the head of the Department of Neurophysiology of the C. I. Z., in whose laboratory the metabolic measurements have been made; to Dr. F. Vargas of the C. I. Z. for useful suggestions; to Mr. A. Escarate, head of the photographic laboratory of the C. I. Z., for excellent collaboration; to Dr. A. Pisano of the University of Cuyo, Mendoza, for his help in collecting the animals, and to Miss A. Garate, of the University of Cuyo, for reading the manuscript. Above all, the author wishes to express his gratitude to Prof. Th. Dobzhansky, of Columbia University, New York, for his suggestions and criticisms.

LITERATURE CITED

CEI, J. M. 1956. Observaciones geneticas preliminares en poblaciones de anfibios argentinos. Biologica, **22**: 45–49.

KIRK, R. L. AND L. HOGBEN. 1946. Studies on temperature regulation II. Amphibia and reptiles. Journal of Experimental Biology, **22**: 213–220.

HEMOLYMPH POLYMORPHISM IN A MOTH AND THE NATURE OF SEX-CONTROLLED INHERITANCE[1]

G. STEHR

Forest Insect Laboratory, Sault Ste. Marie, Ontario

Received December 29, 1958

Campbell (1953) reported that the male pupae of *Choristoneura pinus* Free. are always yellow and female pupae are always green whereas both sexes of *C. fumiferana* (Clem.) may be either yellow or green. This strict sexual dimorphism of the one species and the polymorphism of the other with respect to the same character suggested a common genetic basis, and a study of this promised to shed more light on the population genetics and evolutionary relationships between species and subspecific groups in the genus *Choristoneura*.

It has been established by Junge (1941), Okay (1945), Goodwin and Srisukh (1951), and Goodwin (1952, 1953) that the green colors found in the hemolymph and integument of Lepidoptera and Orthoptera are the result of a combination of yellow carotenoid-protein complexes and blue bile pigments. The blue pigments do not merely appear to be chlorophylls, absorbed from the food, as had been maintained by Poulton (1893) and was still assumed by Gerould (1926), but are products of the insect's own metabolism (Okay, 1953). Little is as yet known about the biochemical and physiological function of these bile pigments, but they have been found as prosthetic groups of important chromoproteins (catalases, peroxidases) (C. H. Gray, 1953). Hidaka (1956) found that during the prepupal stage a neurohormonal mechanism is involved in the determination of a brown-green alternative in pupae of Papilionidae. Nickerson (1956) described a humoral control system in the Desert Locust whereby two hormones determine by their relative balance the phase differences between *solitaria* and *gregaria* forms, which are distinguished by the presence or absence of bile pigments.

Presence or absence of bile pigment in insect hemolymph, whether in conjunction with other pigments or alone, is indeed a frequent requisite of dichromatism or polymorphism. For Lepidoptera, Geyer (1913) gave a long list of species sexdimorphic for lymph coloration, Gerould (1921) noted a polymorphism of lymph color not limited to sex in *Pieris* larvae, and recently P. H. Gray (1953) observed it for Pieris pupae. Phase differences have also been reported in Lepidoptera by Faure (1943a,b), and are suspected to be similarly indicative of population density levels as they are in locusts. It may be mentioned that Gerould's (1921) blue-green *Colias* larvae obviously depended on a gene that affected the yellow carotenoid complex in the lymph but left the blue bile pigments undisturbed. The eggs of the polymorphic *Choristoneura* species are generally green and are probably supplied with this pigment, regardless of whether the mother's hemolymph contained visible amounts of it at the time of scoring. The general occurrence of these bile pigments together with their great variability in response to ecological, physiological, or genetic conditions certainly points to some significant role in developmental and physiological processes.

METHODS AND MATERIALS

The character in question is the color of the hemolymph as seen in a freshly

[1] Contribution No. 514, Forest Biology Division, Research Branch, Canada Agriculture, Ottawa, Canada.

moulted pupa, where the cuticle itself is still unpigmented. For the purpose of this study the simple scoring into two classes, green or yellow, has been found satisfactory, although there are variations of intensity and tinge within the two groups. Fresh pupae may actually be of several shades from a pale, waxy cream to a strong orange-yellow in the "yellow" group; or from a pale, faintly bluish gray, through a brilliant grass-green, to blue-green or turquoise in the "green" group. Comparing with Munsell's (1942) color notation under "Daylight" fluorescent lights, the yellow class comprises hues (H) between 20.0 and 25.0, and the green class hues between 30.0 and 50.0. In both classes the color values (V) may be 7 and 8 or higher, and chroma (C) may range from 4 to 12. In the intermediate range of hues, i.e. around Munsell's chart for 27.5, the two classes blend into each other and differentiation may become difficult; fortunately the frequencies in this range are low. However, scoring errors do occur in both directions. There are also other reasons for such errors: yellow pupae may be scored as green because the early development of black melanin in the cuticle may give a greenish cast, or faintly green pupae may be scored as yellow if the melanic pigments of the chitin are brown or reddish-brown. The best assessment of color is obtained during the first six to ten hours of the pupal period and becomes less certain thereafter.

The laboratory stocks on which the analysis of color inheritance has been based, were all reared under controlled temperature (21° C.) and humidity (70% R.H.) by a standard technique (Stehr, 1954), using deep-frozen spring shoots of balsam fir, *Abies balsamea* (L) Mill., as food. They originated from collections taken in northern and northwestern Ontario. For the assessment of phenotypic frequencies of pupal color, field collections of fifth- and sixth-instar larvae were shipped to the laboratory in the three years 1953 to 1955 and reared to pupation. The eastern spruce budworm, the taxonomic type of *C. fumiferana,* was obtained from Newfoundland, New Brunswick, Quebec, Ontario, and Manitoba; collections from Alberta and British Columbia represent the western one- and two-year-cycle budworm populations which are considered at the present time to be subspecific groups of *C. fumiferana,* completing their life cycle either in one year, like the eastern form of the species, or requiring two years with two overwintering diapause periods. Collections of the jack-pine budworm, *C. pinus,* were obtained from northwestern Ontario and southern Manitoba. For the most recent taxonomic review see Freeman (1958).

Observations

Analysis of Color Inheritance in the Eastern Form of *C. fumiferana*

The data obtained by mating animals with green and yellow pupal hemolymph are presented in table 1. As the segregation ratios in many families are not the same in the two sexes, a sex-linked factor must be involved. Sex-linked factors alone, however, cannot account for segregation ratios other than 1:1 in females; as Smith (1944) has shown that males are homogametic, females will be heterogametic, as is generally the case in Lepidoptera, carrying only one X-chromosome derived from their father. Hence female sibships are uniform with regard to sex-linked characters if the male parent was homozygous, or with a heterozygous father, they will segregate into two equal parts.

Obviously, since many female segregations do not fit a 1:1 ratio autosomal genes must also be involved. However, most of the ratios that deviate from equality are either 1:3 or 3:1, so that it should be sufficient to assume one autosomal and one sex-linked gene only. This conclusion is substantiated by the occurrence of the ratios 1 yellow : 1 green in females and 7 yellow : 1 green in males, which can only be interpreted as derivative of a dihybrid segregation where one of the two gene loci is sex-linked and the female sex is heterogametic. In such a dihybrid cross, these segregation ratios must have been obtained from the mating of an autosomally heterozygous female with a doubly heterozygous male, both phenotypically yellow. Six genotypes of each sex must be produced by such a mating, with the frequencies 1 : 1 : 2 : 2 : 1 : 1, and in males only one eighth is green, in females one half. In both sexes one of the two central groups with a double

TABLE 1. *Segregations in families of the eastern form of* C. fumiferana

In this as well as in table 2, families are numbered to show their relationships by means of a decimal notation whereby each place corresponds to a generation of brother-sister matings in direct lineage starting with a letter. One letter indicates the original mating of two genetically unknown individuals collected as larvae in the field; double letters indicate crosses between laboratory reared families. In this and in all subsequent tables bracketed numbers are considered to be scoring errors. Theoretical ratios are given on the basis of separate denominators for each sex. A, B, D: derived from Indian Lake (Vermilion Bay), Ont., 1952. C, E, G, I: from Indian Lake, Ont., 1955. P: from Sioux Lookout, Ont., 1955. Q, R: from Spruce Woods Forest Reserve, Man., 1953. K, O, N: laboratory families, derived in 1950 from Quibell, Ont.

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ green × ♂ green		A334	0	9	4	2
		C1	0	0	3	2
		C3	(2)	6	7	6
		D	0	12	2	2
		D3	0	22	12	11
		D4	0	26	4	12
		G4	0	13	6	8
		G21	0	9	3	4
		G22	0	30	16	8
		G41	(1)	11	7	5
		G42	0	7	6	7
		K	0	10	3	3
		O1	0	2	2	2
	0 : 1/1 : 1		(3)	155	73	70
♀ green × ♂ green		G3	4	4	3	6
		I1	2	1	2	2
		J5	2	5	0	5
		K2	3	13	4	13
		ON45	4	11	4	8
		ON91	9	36	8	23
		ON92	2	5	3	5
		OH93	6	20	9	13
		ON94	2	4	1	6
		ON911	0	2	2	5
		Q	0	5	4	10
		Q1	1	2	3	9
		R	2	4	4	13
		R2	1	4	1	5
	1 : 3/1 : 3		38	116	48	123
♀ green × ♂ green		C7	0	3	0	7
		D1	0	1	0	1
		G1	0	10	(1)	20
		G2	0	3	0	2
		G43	0	11	(1)	8
		O	0	19	0	13
		O2	0	2	0	2
		Q2	(1)	9	(1)	9
		Q21	0	2	0	7
	0 : 1/0 : 1	[illegible]	(1)	61	(3)	69
♀ green × ♂ yellow		A32	4	4	11	8
		A331	0	0	1	1
		A342	5	5	6	2

TABLE 1. *Segregations in families of the eastern form of* C. fumiferana—(*Continued*)

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ green × ♂ yellow		C	19	28	10	12
		E	3	4	4	4
		E1	3	3	1	5
		K1	11	10	11	8
		ON2	10	14	18	17
		ON3	29	17	22	16
		ON4	11	15	21	16
		ON4311	2	3	9	4
		ON5	8	8	6	8
		ON51	9	11	10	4
		ON6	4	3	3	5
		ON7	19	14	18	15
	1 : 1/1 : 1		137	139	150	125
♀ green × ♂ yellow		A35	2	4	5	0
		A8	13	8	31	(1)
		A9	17	13	34	(1)
	1 : 1/1 : 0		32	25	70	(2)
♀ green × ♂ yellow		D1	0	1	0	1
		G44	0	4	0	3
		ON1	0	20	0	15
		ON8	0	50	(3)	40
		ON9	(3)	47	(6)	30
	0 : 1/0 : 1		(3)	122	(9)	89
♀ green × ♂ yellow		A34	2	5	4	11
		E2	1	4	3	8
		G	0	12	2	11
		G5	3	6	4	8
	1 : 3/1 : 3		6	27	13	38
♀ green × ♂ yellow		A33	8	17	12	6
		A6	9	18	38	7
		A7	0	1	5	1
		B	8	16	10	3
		E3	3	10	7	1
		G52	0	0	3	0
		G53	0	0	2	1
	1 : 3/3 : 1		28	62	77	19
♀ green × ♂ yellow		I	33	13	37	9
		P	5	2	6	1
	3 : 1/3 : 1		38	15	43	10
♀ yellow × ♂ green		A5	6	6	17	(1)
		I2	4	1	6	0
	1 : 1/1 : 0		10	7	23	(1)

TABLE 1. *Segregations in families of the eastern form of* C. fumiferana—(*Continued*)

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ yellow × ♂ green		A341	0	43	30	19
		C2	(1)	6	7	5
	0 : 1/1 : 1		(1)	49	37	24
♀ yellow × ♂ green	0 : 1/0 : 1	C6	0	7	(1)	16
♀ yellow × ♂ green		L	6	13	20	7
		P1	5	9	13	5
	1 : 3/3 : 1		11	22	33	12
♀ yellow × ♂ green		ON43	13	7	5	13
		ON431	6	4	7	5
		ON4312	9	6	7	6
		ON913	1	3	0	0
		ON914	8	3	5	2
		ON52	3	3	7	6
	1 : 1/1 : 1		40	26	31	32
♀ yellow × ♂ yellow		A	27	24	37	5
		A3	8	10	14	0
		A4	13	14	21	4
		C4	2	2	5	1
		G51	14	15	13	4
		I3	4	3	7	1
	1 : 1/7 : 1		68	68	97	15
♀ yellow × ♂ yellow		A1	14	0	10	0
		A31	33	0	34	0
		A311–8	122	0	105	0
		C5	36	(1)	53	(1)
		C51–3	7	(1)	9	0
		I4	3	0	3	0
		N	46	0	48	0
		N1–12	83	0	91	0
		ON31	3	0	4	0
		ON41–2	29	0	36	(1)
		ON53	7	0	13	(2)
		ON71	6	0	7	0
		ON912	15	0	17	0
		P2	5	0	6	0
	1 : 0/1 : 0		409	(2)	436	(4)
♀ yellow × ♂ yellow		A2	2	2	11	0
		A333	1	2	1	0
	1 : 1/1 : 0		3	4	12	0

frequency represents the parental genotype and is, therefore, yellow; in females, the other central group is also heterozygous at the autosomal locus but must carry the opposite sex-linked allele and thereby becomes green; if it were yellow, it would have to be concluded from the proportion of yellow females, that in this sex heterozygosity at the autosomal locus is the necessary and sufficient condition for yellow phenotype. This is not so, however, as true breeding yellow families would then be impossible, whereas they are, in fact, frequently found. Therefore, the phenotype of autosomally heterozygous females is controlled by the type of allele carried on their X-chromosome.

There are four more genotypes in the female offspring of this cross; all are homozygous at the autosomal locus, two for each of the alternative alleles. Each homozygote occurs with one or the other sex-linked allele respectively. To satisfy the segregation proportions, two of these four genotypes are yellow, two are green. The decision about the phenotype cannot now be assigned to the two sex-linked alleles, as the character would then be simply sex-linked. These four genotypes, therefore, segregate according to their autosomal alleles.

It might be concluded that the decision about the phenotype is primarily made by an autosomal homozygote, while the heterozygotes are segregating according to a sex-linked switch gene. This seems undoubtedly to describe the situation in females. For the homogametic males, with their segregation ratio of 7 yellow : 1 green, a double homozygote might be assumed as the genotype of the green segregant. However, the males can be homozygous at the sex-linked locus only for the allele that caused their mother to be yellow; for this allele half the males are homozygous, the other half heterozygous. If the autosomal locus, presumably homozygous for the "green" allele, can override the opposing effect of a homozygous sex-linked locus, it should also override that locus in its heterozygous phase, making the male ratio a simple 1 : 3. This leads to the conclusion that the green male segregant is autosomally homozygous for "green," but is able to express this phenotypically only where the sex-linked locus is not homozygous for "yellow." On this basis, the genetic mechanism producing the hemolymph polymorphism in this species can be assumed to consist of two alleles at an autosomal locus responsible for the production or occurrence of blue bile pigment and thus green hemolymph (symbols: B/b), and a pair of sex-linked suppressor alleles, preventing such pigments from occurring or from becoming visible (symbols: Su-B/su-B). The observed data can be satisfactorily interpreted according to the scheme presented in figure 1.

We are thus dealing with a case of sex-linked

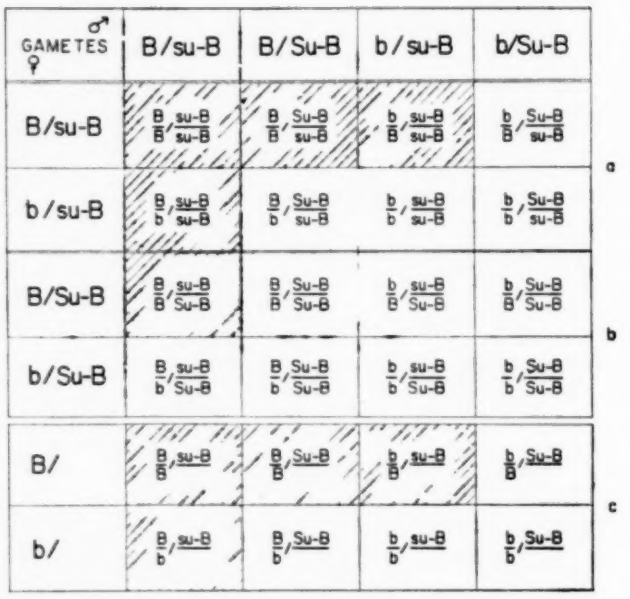


FIG. 1. Diagram of 1:1/1:1 and 1:1/7:1 ratios obtained by mating the doubly heterozygous male to the two autosomally heterozygous females: a) male offspring if mother carried the recessive; b) if it carried the dominant suppressor allele on the X-chromosome; c) female offspring, identical in both cases. Open = yellow, hatched = green phenotoypes.

epistasis, distinguished by the fact that the expression of the epistatic gene is not dependent merely on the presence or absence of its dominant allele but is dependent on the balance between the number of dominant autosomal hypostatic alleles to the number of dominant sex-linked epistatic alleles. The interaction between the alleles of the two gene loci is such that wherever the number of sex-linked dominants is equal to or exceeds the number of autosomal dominants, the hemolymph is yellow; where the sex-linked dominants are in a minority, the hemolymph is green.

Figure 2 shows the possible genotypes for both sexes in *C. fumiferana*. There are six female genotypes, three yellow and three green, and nine male genotypes, six yellow and three green. The figure further shows the segregation ratios that will result from any mating combination. Most of these ratios have been recovered in rearings (table 1).

Pupal color in the western spruce budworm

Table 2 summarizes data of pupal color segregation found in rearings of western one-year cycle budworm. Not as many different segregation ratios are realized here as there were in the eastern form. The body of data is rather small as it was considerably more difficult to maintain this material in laboratory stocks on *Abies balsamea*, which is not their normal host. Therefore, a larger proportion of the data consists of families obtained from field-collected parents, thus making the recovery of all possible

♀ \ ♂	B/B su-B/su-B	B/B Su-B/su-B	B/b su-B/su-B	B/B Su-B/Su-B	B/b Su-B/su-B	B/b Su-B/Su-B	b/b Su-B/Su-B	b/b Su-B/su-B	b/b su-B/su-B
B/B Su-B/Su-B	0:1 / 0:1	0:1 / 1:1	0:1 / 1:1	0:1 / 1:0[J]	1:3 / 3:1	1:1 / 1:0	1:0 / 1:0	1:1 / 1:0	0:1 / 1:0
B/B su-B/Su-B	0:1 / 0:1	0:1 / 0:1	0:1 / 0:1	0:1 / 0:1	1:3 / 1:3	1:1 / 1:1	1:0 / 1:0	1:1 / 1:1	0:1 / 0:1
B/b su-B/Su-B	0:1 / 0:1	1:3 / 1:3	1:3 / 1:3	1:1 / 1:1	1:1 / 1:1	3:1 / 3:1	1:0 / 1:0	3:1 / 3:1	1:1 / 1:1
B/b Su-B/Su-B	0:1 / 1:1	1:3 / 3:1	1:3 / 3:1	1:1 / 1:0	1:1 / 7:1	3:1 / 1:0[D]	1:0 / 1:0	3:1 / 1:0	1:1 / 1:0
b/b Su-B/Su-B	0:1 / 1:0	1:1 / 1:0	1:1 / 1:0	1:0 / 1:0	3:1 / 1:0	1:0 / 1:0	1:0 / 1:0	1:0 / 1:0	1:0 / 1:0
b/b su-B/Su-B	0:1 / 0:1	1:1 / 1:1	1:1 / 1:1	1:0 / 1:0	3:1 / 3:1	1:0 / 1:0	1:0 / 1:0	1:0 / 1:0	1:0 / 1:0

FIG. 2. Segregation ratios for hemolymph color expected in matings between the six female and nine male genotypes responsible for the polymorphism in *C. fumiferana*. For explanation of the cells indicated D and J see text. Open = yellow, hatched = green parents.

segregation ratios dependent on the frequency of natural occurrence of some genotypes; it will be shown later that some of these must be rare. Finally, some ratios may be distorted by scoring errors, which are likely to be more frequent here as the pupa of this form has a distinctly different pigmentation of the cuticle from that of its eastern counterpart; considerably more melanic pigment is deposited more rapidly and in a diffuse distribution, whereas in the eastern type distinct patterns are laid down between the veins of the pupal wing pad.

Despite these shortcomings it can be stated that all the segregation ratios found so far can be accounted for by the same genetic mechanism that operates in eastern *C. fumiferana*, and the table has accordingly been arranged and grouped so that the data are compared to ratios that might be expected in stocks of eastern origin from parents of the same color classes.

If the conclusion is correct that the genetic basis for hemolymph polymorphism is identical in the eastern and western populations, no disturbances should be expected in crosses between them. Table 3 shows the outcome of such crosses and, with the exceptions to be discussed presently, there is indeed no contrary evidence. The exceptions are two of the larger families, indicated by asterisks in table 3, that were obtained by mating eastern green females to western yellow males. Referring to figure 2 it will be seen that ordinarily such matings can give only 1 : 0, 1 : 3, 3 : 1, or 1 : 1 segregations in males. Both families obviously agree with a 1 : 1 segregation. If this is the case, however, females are likewise required to segregate 1 : 1. Statistically, the data disagree strongly with this, but before the assumption of identical gene mechanisms for lymph color determination in the two subspecies can be questioned, it must be noted that the sex-ratio in these two families, as indeed in two others obtained from yellow × yellow matings, is significantly disturbed by a deficiency of females. This indication of some hybrid inviability in the heterogametic sex, in accordance with Haldane's rule, may support the taxonomic separation of the two subspecies. Possibly, some incompatibility between their genetic constitutions resides in X-chromosomes derived from the western form and is connected or linked with some of the alleles responsible for hemolymph pigmentation. This situation should prove a promising starting point to clarify the extent of genetic differences between the two groups. At the same time, the conclusion that the color polymorphism is based on a gene system similar in basic nature in both subspecies is certainly justified.

Crosses between *C. fumiferana* and *C. pinus*

As mentioned before, there is no obvious polymorphism of pupal color in *C. pinus;* instead, we find a strict sexual dimorphism. The main purpose of the interspecific crosses was to ascertain whether this dimorphism and the polymorphism in *C. fumiferana* are based on a similar epistasis mechanism, as is suggested by the fact that such a dimorphism may be expected in *C. fumiferana* in a stock of double dominant homozygotes (see fig. 2, the cell indicated "J").

The data of table 4 show, first, the great paucity of progeny in these hybrid families. *C. pinus* has been hardly adaptable to laboratory rearing, and this difficulty is reflected here. The situation renders any interpretation on the basis of statistical probabilities at present impossible and only the occurrence or non-occurrence of segregant classes may serve for deductions.

TABLE 2. *Segregations in families of the western one-year cycle form of* C. fumiferana
Families E, D, L, I, M, H, J: derived from Lillooet, B. C. 1953.
A, B: from Clinton, B. C. 1957.

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ green × ♂ green		A	0	20	(2)	37
		HI11	0	2	0	1
			—	—	—	—
	0 : 1/0 : 1		0	22	(2)	38
♀ yellow × ♂ green		K	3	1	7	(1)
		LM24	11	5	9	(1)
			—	—	—	—
	1 : 1/1 : 0		14	6	16	(2)
♀ yellow × ♂ green	1 : 3/3 : 1	HI1	7	12	20	9
♀ yellow × ♂ green	1 : 1/1 : 1	L2	9	6	7	5
♀ yellow × ♂ green	0 : 1/1 : 1	E	0	4	3	2
♀ green × ♂ yellow		D	2	5	4	2
		D1	3	1	2	4
		ED1	4	6	12	7
		L	9	5	6	5
		K1	5	3	4	6
			—	—	—	—
	1 : 1/1 : 1		23	20	28	24
♀ green × ♂ yellow		L1	21	6	9	7
		LM241	5	3	3	1
			—	—	—	—
	3 : 1/3 : 1		26	9	12	8
♀ green × ♂ yellow	1 : 3/1 : 3	I	0	3	1	3
♀ yellow × ♂ yellow		B	23	0	26	(1)
		M	6	0	13	0
		LM1	39	0	35	(1)
		LM21	2	0	2	0
		LM22	4	0	0	0
		LM23	10	(1)	9	0
		H	7	(1)	3	(1)
		H1	16	0	21	0
		H12	17	(1)	22	(3)
		H121	12	(2)	9	(1)
			—	—	—	—
	1 : 0/1 : 0		136	(5)	130	(7)
♀ yellow × ♂ yellow		LM2	13	2	8	1
		J	3	0	7	3
		JD1	13	7	15	8
			—	—	—	—
	3 : 1/3 : 1		29	9	30	12

Females have occasionally been scored as yellow, but their frequency hardly exceeds the limits of error; in general they are green, regardless of the type of mating that produced them, and in most families males are yellow. In males of some families, however, segregation certainly occurs, e.g. in the first category in table 4, ♀ *fumiferana* green × ♂ *pinus* yellow.

As there is no reason to suspect any heterozygosity in the genetic mechanism for lymph color in *C. pinus,* the segregation of hybrids should be accountable for by a heterozygous *fumiferana* parent, and there is only one such heterozygous green female in *C. fumiferana,* that with the formula B/b;su-B/– (see fig. 2). The segregation in the male offspring reflects, then, the difference between the two alleles at the B locus in *C. fumiferana,* with the green males presumably being of the formula $B/+^{pi}$; su-$B/+^{pi}$ where $+^{pi}$ symbolizes for the time being the *pinus* alleles at the corresponding loci, regardless of their exact nature.

A similar segregation, e.g. family C in table 4, occurred in the reciprocal crosses ♀ *pinus* green × ♂ *fumiferana* green; here also the segregation of male offspring must be accounted for by heterozygosity of the *fumiferana* parent. Although either of two types of males, B/b; su-B/su-B or B/B; Su-B/su-B, may have been used, a 1 : 1 segregation in males is expected in each case, the green ones being of the same genetic constitution, $B/+^{pi}$; su-$B/+^{pi}$, as the males discussed above.

Quite clearly, the gene system that normally causes all males in *C. pinus* to be yellow breaks down if it is combined with the arrangement of mutant and suppressor alleles from *C. fumiferana* that is weighted in favor of the autosomal pigment protagonist. In *C. pinus,* also, some epistatic relationship therefore exists between an autosomal protagonist and an X-chromosomal antagonist, the latter as strong or stronger than the former in males, and weaker in females, and it is this that controls the sexual dimorphism. It is important to realize that the dimorphism is a matter of two

TABLE 3. *Crosses between the eastern and the western one-year cycle form of* C. fumiferana

The letter combinations DB or BD in front of the family letter indicate the direction of the cross. B for *C. fumiferana* eastern form (balsam fir), D for the western form (Douglas fir).

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ *fumiferana* western × ♂ *fumiferana* eastern						
♀ yellow × ♂ yellow		DB–A	7	(1)	5	0
		DB–B	12	(1)	9	0
		DB–C	3	0	1	0
	1 : 0/1 : 0		22	(2)	15	0
♀ yellow × ♂ green	0 : 1/1 : 1 or 1 : 3/1 : 3	DB–D	0	6	2	4
♀ green × ♂ green	0 : 1/0 : 1	DB–E	0	12	(1)	7
♀ *fumiferana* eastern × ♂ *fumiferana* western						
♀ yellow × ♂ green	1 : 1/1 : 1	BD–A	6	3	5	5
♀ green × ♂ yellow		BD–B	13	18	21	13
		BD–C*	6	14	18	17
		BD–D*	4	16	12	17
	1 : 1/1 : 1		23	48	51	47
♀ yellow × ♂ yellow		BD–E	9	0	6	0
		BD–F*	15	0	28	(1)
		BD–G*	16	0	28	(2)
		BD–H	7	0	6	0
		BD–I	5	0	12	0
	1 : 0/1 : 0		52	0	80	(3)

* Families with sex-ratios significantly deviating from .5.

TABLE 4. *Segregations in crosses between the eastern form of* C. fumiferana *and* C. pinus

The letter combinations BJ and JB in front of the family letter indicate the direction of the cross. B for *C. fumiferana* eastern form, J for *C. pinus* (jack pine).

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
♀ *fumiferana* green × ♂ *pinus*		BJ–G	0	0	1	0
		BJ–K	0	17	7	5
			0	4	2	1
		BJ–E	0	8	4	1
			0	8	1	4
			—	—	—	—
	0 : 1/1 : 1		0	37	15	11
♀ *fumiferana* yellow × ♂ *pinus*			(2)	13	12	0
			0	1	2	0
			0	0	1	0
		BJ–D	(1)	2	2	0
			0	0	1	0
		BJ–F	(1)	6	5	0
			0	0	1	0
			0	1	0	0
			0	1	1	0
			—	—	—	—
	0 : 1/1 : 0		(4)	24	25	0
♀ *pinus* × ♂ *fumiferana* green		JB–H	0	0	2	0
			0	0	0	1
			0	0	0	1
		JB–C	(1)	4	1	3
			—	—	—	—
	0 : 1/1 : 1		(1)	4	3	5
♀ *pinus* × ♂ *fumiferana* yellow		JB–B	0	3	8	0
		JB–A	0	15	16	0
			0	0	2	0
		JB–J	(1)	5	4	(1)
			0	3	0	0
			(1)	3	1	0
		JB–I	0	1	4	0
			—	—	—	—
	0 : 1/1 : 0		(2)	30	35	(1)

specific loci, not one of a "genetic background" difference between the two sexes, as is shown by the effect of a suitable allele replacement in the green F_1 males.

It remains to be decided whether the B allele or the su-B allele of *C. fumiferana*, or both together, are responsible for upsetting the usual balance between the corresponding *pinus* alleles. In other words, are half the F_1 males green because of a substitution at the autosomal or at the X-chromosomal locus? It may be noted that should the change have occurred as a mutation in *C. pinus*, this would have appeared as an autosomal or a sex-linked mutation, in either case dominant and sex-limited to males, because females remain green as if no change had taken place. Turning to a consideration of the F_1 females, the cross ♀ *fumiferana* green × ♂ *pinus* yellow (the first group in table 4), with the female an autosomal heterozygote as concluded before, two female genotypes are expected, $B/+^{pi};+^{pi}/-$ and $b/+^{pi};+^{pi}/-$, both of which are green. If this result is compared to the *fumiferana* genotypes in figure 2, no doubt can exist that one, the other, or both of the alleles that constitute the epistatic balance system in *C. pinus* are different from those of the *fumiferana* system. The possibility that the genetic mechanism of *C. pinus* differs from that of *C. fumiferana* only in that it lacks the heterozygosity at the two epistatic loci that are responsible for the polymorphism of the latter has

now to be discarded. *C. pinus* is not merely homozygous, as a *fumiferana* stock of the constitution indicated in cell "J" of figure 2 would be; the difference between the two species is not only one of different gene frequencies, but also one of different gene potencies.

Polymorphism Resulting from Potency Differences in Epistatic Systems

Gene potencies serve to describe, in the form of a numerical model, the dominance and epistasis relationships between different alleles at the loci involved, so that the phenotypic outcome of their interaction can be expressed consistently for any of their combinations. The simplest way of describing two alleles at the same locus is to ascribe a smaller and a larger number to them; the relationship of epistasis between two loci can then be expressed by the difference resulting from the subtraction of the sum of the values ascribed to the alleles at the antagonistic suppressor locus from the sum of allelic values at the protagonistic locus. Alternative phenotypes are indicated by the sign of this difference, zero or minus indicating the prevalence of the antagonist or absence of the character, whereas plus values indicate prevalence of the protagonist or presence of the given character. Series of phenotypes may be described, as well, by omitting the assumption of a threshold value at zero or by assuming more than one threshold for more than two alternative phenotypes. By the proper choice of the allelic potency values, a large number of relationships and some general genetic phenomena can thus be described in a unified form. Such a numerical model is, however, intended as nothing more than a formal expression of qualitative phenotypic differences based on quantitative relationships. The choice of simple addition as the *modus operandi* for this model neither implies nor even suggests that allelic action is actually additive; multiplication provides the same end result.

Figure 3 gives the values that properly express the situation in *C. fumiferana*. This is the lowest possible set of whole numbers that will give the desired results. To this has to be fitted a set of potency

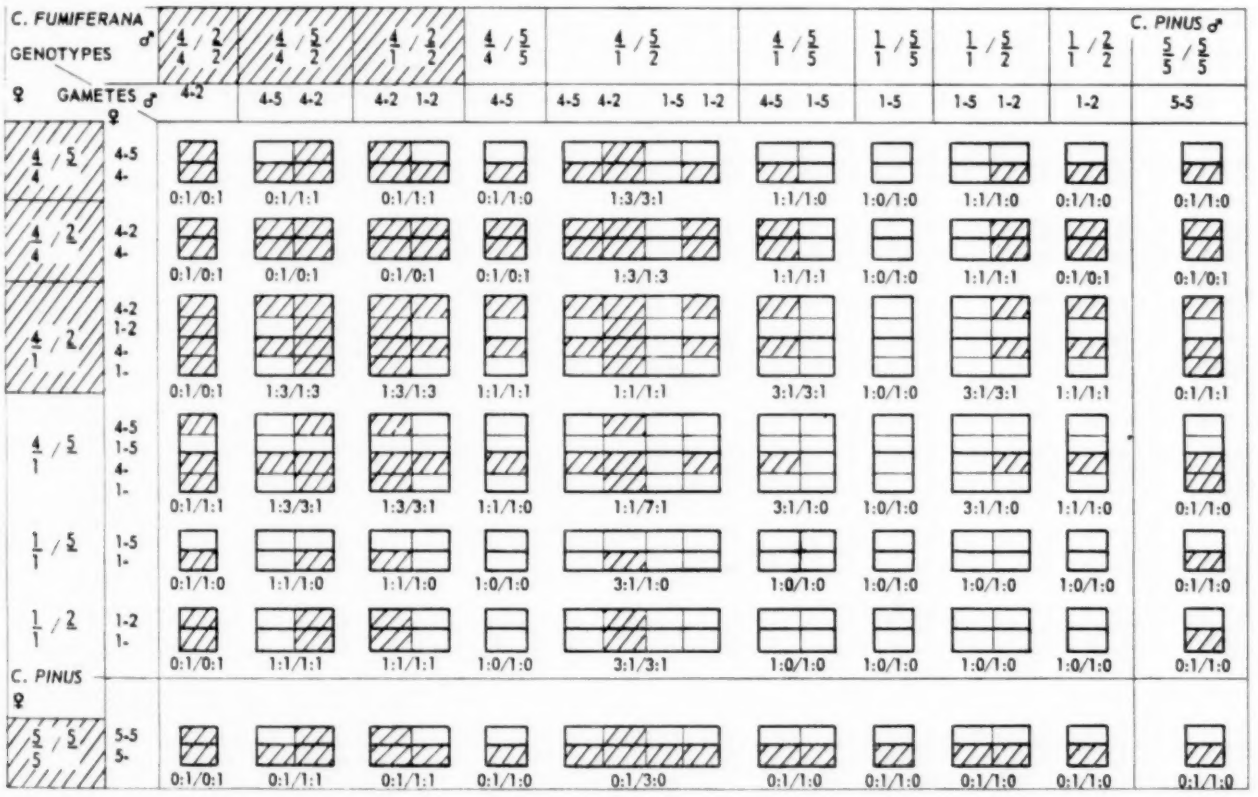


FIG. 3. Interpretation of the sex-controlled polymorphism in *C. fumiferana*, the sexual dimorphism in *C. pinus* and the segration ratios of their hybrids by an allelic potency notation for the two antagonistic loci involved. Open = yellow, hatched = green pupal hemolymph.

TABLE 5. *Segregations in F_2 and backcross families between* C. fumiferana *and* C. pinus

The family numbers relate these F_2 and backcross families to the F_1 families shown in table 4. E.g. four successful brother-sister matings have been obtained from family JB–A of table 4, JB–A1, JB–A2, etc. The backcross family BBJ–G1 is the result of mating a green *C. fumiferana* female with the male of BJ–G in table 4. The expected ratio is based on the potency formulation of the mating types, explained in the text.

Mating type	Expected ratio	Family no.	♀♀ yellow	♀♀ green	♂♂ yellow	♂♂ green
F_2-families (sib matings)						
♀ green × ♂ yellow						
1/5–2/– × 1/5–2/5	1 : 3/7 : 1	JB–A1	1	4	9	1
1/5–2/– × 1/5–5/5	1 : 3/3 : 1	JB–A3	0	4	4	1
		JB–A2	1	2	8	0
		JB–A4	1	2	5	0
		JB–B1	1	1	0	0
		JB–B2	1	0	3	0
1/5–5/– × 1/5–5/5		JB–B3	1	3	7	0
or			—	—	—	—
1/5–5/– × 4/5–5/5	1 : 3/1 : 0		5	9	23	0
4/5–5/– × 4/5–5/5	0 : 1/1 : 0	JB–C3	0	0	8	(1)
♀ green × ♂ green						
		JB–C1	0	9	7	10
		JB–C4	0	0	1	1
			—	—	—	—
4/5–5/– × 4/5–2/5	0 : 1/1 : 1		0	9	8	11
4/5–2/– × 4/5–2/5	0 : 1/0 : 1	JB–C2	0	4	0	12
			35 ♀♀		78 ♂♂	
Backcrosses to *fumiferana* ♀♀						
♀ green × ♂ yellow						
4/4–2/– × 4/5–5/5	0 : 1/0 : 1	BBJ–D1	0	10	(1)	13
	—	BBJ–E1	0	2	1	0
	—	BBJ–E2	0	1	1	1
	—	BJB–I1	0	0	1	0
	—	BJB–I2	0	2	2	0
	—	BJB–J2	0	1	1	0
4/4–2/– × 1/5–2/5	1 : 3/1 : 3	BBJ–E3	0	3	1	3
		BBJ–F1	1	4	5	6
		BJB–H1	1	5	3	3
			—	—	—	—
1/4–2/– × 4/5–5/5	1 : 3/1 : 1		2	9	8	9
4/4–5/– × 1/5–2/5	1 : 3/3 : 1	BBJ–G1	5	13	17	5
♀ green × ♂ green						
4/4–5/– × 4/5–2/5	0 : 1/1 : 1	BBJ–E4	0	10	2	4
♀ yellow × ♂ yellow	—	BJB–J2	1	0	1	0
			59 ♀♀		71 ♂♂	

values for the alleles at the equivalent loci of *C. pinus* so that they express the dimorphism of the pure species as well as the phenotypes of the resulting hybrids. With regard to the dimorphism, it is clear that any set of values by which an antagonistic allele is made equal to or larger than a protagonistic one fulfills the requirement, provided that it remains smaller than the sum of two protagonistic alleles. This can be written as $A < X < AA$ where A means the value of an autosomal protagonistic allele and X that of the X-chromosomal antagonist. By tautology, this expression can also be written as $X < AA < XX$ and is then equivalent to a frequently used expression for the balance theory of sex determination.

The segregation ratios encountered in some hybrid F_2 and backcross families can best be explained by the assumption of a potency of 5 at both the protagonistic and antagonistic loci in *C. pinus*. It is this value that has been entered as the bottom row in figure 3 to represent *pinus* × *fumiferana* crosses and at the right-hand margin for the reciprocal crosses. As an example of how the assignment of proper potency values indeed permits the consistent interpretation of the available data, the mating "JB-A" of table 4 and an F_2 family derived from it are used in figure 4. The conventional gene symbols are replaced by numerical values, the left member of the genetic formula standing for the autosomal allelic values and the right for the X-chromosomal ones, between which the symbols for relative size indicate the prevalence of one or the other side that determines the phenotype. Table 5 gives all F_2 and backcross data obtained so far together with the potency formulae of the matings that presumably produced the respective segregation ratios. It should be mentioned here again that the crosses are not only difficult to obtain but lead to developmental difficulties, as may be seen in a comparison of sex ratios. The backcrosses of yellow F_1 males to green *fumiferana* females show no disturbance, whereas in F_2 families half the females are missing at pupation; the rearing records show that this differential mortality occurs in the later larval instars. There is no reason to suspect that this mortality affected the segregation classes for lymph color differentially, as it may have in the crosses between eastern and western *C. fumiferana,* but it certainly is a case of interspecific hybrid inviability.

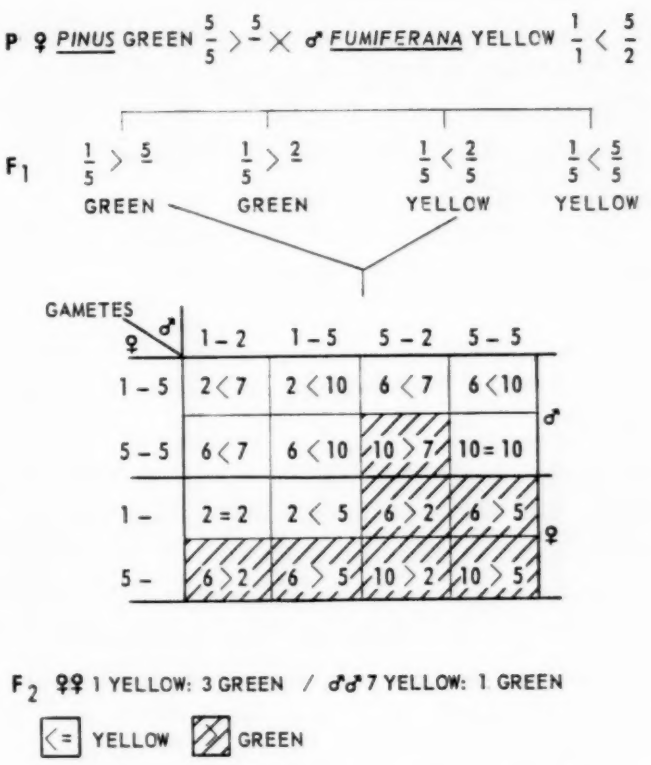


FIG. 4. Interpretation of a *pinus* × *fumiferana* cross by the autosomal–X-chromosomal balance system that determines yellow v. green lymph color.

It will be realized that the values assigned to the *C. pinus* genes in this way express their potencies only relative to those of the *C. fumiferana* alleles. With more information accumulating from more material and, if possible, from larger families, the potency values at present adopted may have to be revised. The same may be true of those for the western forms of *C. fumiferana,* which so far are assumed not to be different from those in the eastern populations, although certain discrepancies connected with survival differentials may, upon further investigation, call for some readjustments or the recognition of the presence of more than two alleles at each locus in some or all

of the natural populations. Several alleles of varying potency have been found by Glass (1957) in a similar mutant-suppressor system, both components of which were located on autosomes.

However, it is not the object of the present paper to establish any final potency values for the epistatic gene systems that are responsible for hemolymph polymorphism in the budworm complex being investigated. The objective is to establish that such a system is at the base of both the polymorphism and the dimorphism and that it provides a unifying principle of interpretation consistent with the facts. If this has been achieved it may be justifiable to trace such evolutionary relationships between the three *Choristoneura* groups as may be deduced on the basis of the presently available information.

Phenotypic Frequencies in Natural Populations

A large number of field populations have been sampled and their phenotypic frequencies for green pupae in both sexes have been plotted in figure 5. In the eastern populations of *C. fumiferana,* the percentages for females and males approximate 70 and 50 respectively. Those for the western 1-year cycle are much lower, 30 or less for females and down to zero for males. The 2-year cycle populations, though low in number, occupy an intermediate position. Referring to figure 2 it will be seen that such percentages might be obtained in a stock that is homozygous for the sex-linked Su-B allele and heterozygous at the autosomal locus (see fig. 2 cell "D"). The position of *C. pinus* is in the right-hand corner, 100% females green and 0% males, for complete sexual dimorphism. As has been shown by its hybrids, the situation here is not merely to be attributed to a shift in gene frequency, but involves also at least one potency change by mutation at the autosomal protagonistic locus.

If a natural population contains two different alleles at the autosomal as well as at the X-chromosomal locus of the

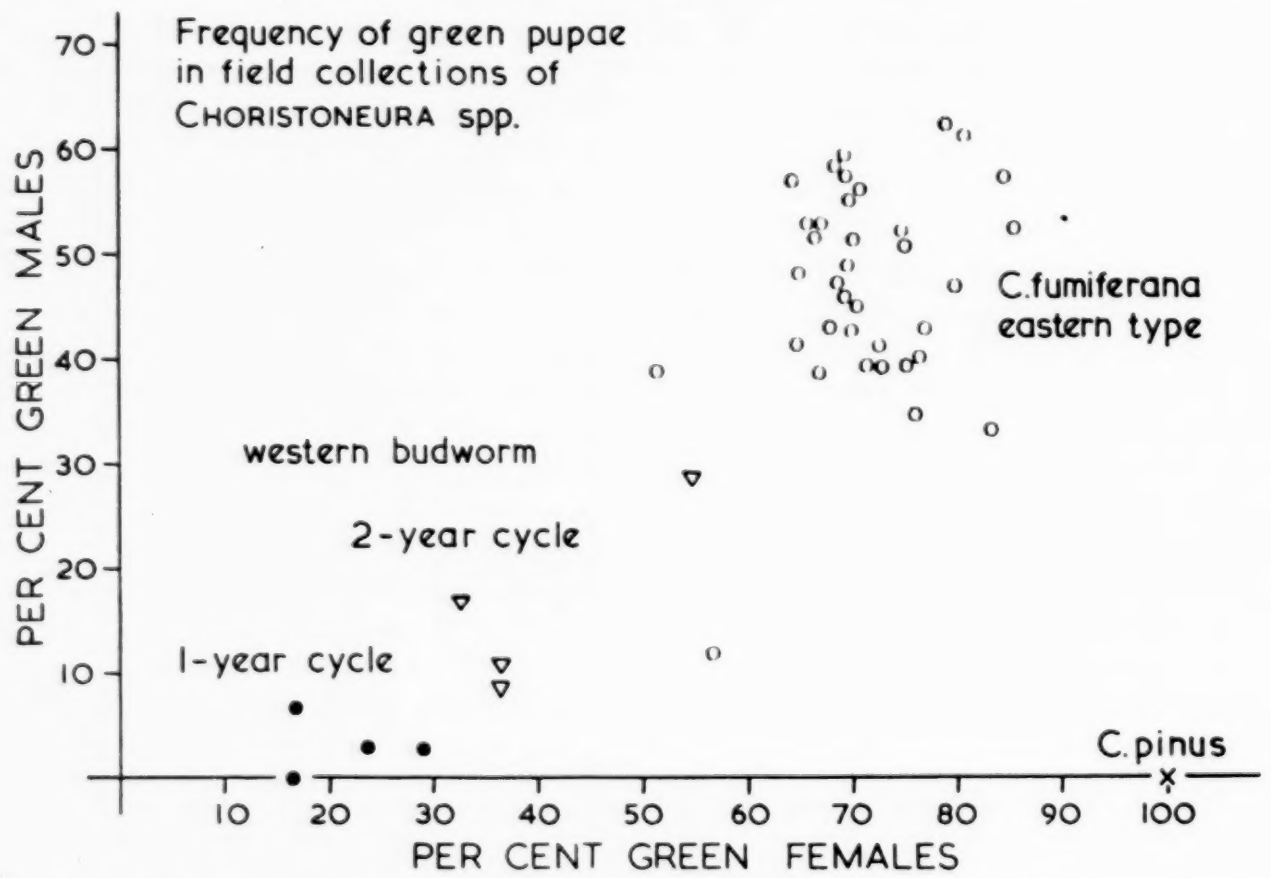


FIG. 5. Samples represented by spots were collected in 1953, 1954, and 1955 in various localities from Newfoundland to British Columbia with only three samples of less than 50 individuals included.

TABLE 6. *Expected phenotypic frequencies in random populations over the range of gene frequencies at the indicated loci, keeping the gene frequency at the other locus constant at .5. * and **: approximate positions of the eastern and the western one-year cycle form of* C. fumiferana

Gene frequencies		Expected phenotypic frequencies	
Autosomal B(4) allele	X-chromosomal Su(5)-B allele	% green females	% green males
0.0	.5	0.0	0.0
.1	.5	10.00	5.25
.2	.5	20.00	11.00
.3	.5	30.00	17.25
.4	.5	40.00	24.00
.5	.5	50.00	31.25
.6	.5	60.00	39.00
.7	.5	70.00	47.25*
.8	.5	80.00	56.00
.9	.5	90.00	65.25
1.0	.5	100.00	75.00
.5	0.0	75.00	75.00
.5	.1	70.00	65.25
.5	.2	65.00	56.00
.5	.3	60.00	47.25
.5	.4	55.00	39.00
.5	.5	50.00	31.25
.5	.6	45.00	24.00
.5	.7	40.00	17.25
.5	.8	35.00	11.00
.5	.9	30.00	5.25**
.5	1.0	25.00	0.0

relative potencies found in *C. fumiferana,* and carries all four alleles with a frequency of 0.5, then, with random breeding, the expected frequencies of phenotypes are 50% of either class in females and 68.75% yellow to 31.25% green in males. This simply follows from the sum of the two characteristic segregation ratios obtained by mating the doubly heterozygous male to the two types of autosomally heterozygous females, distinguished from each other by the alternative X-chromosomal alleles. These two ratios are 1 : 1/7 : 1 from the yellow female and 1 : 1/1 : 1 from the green one, together 1 : 1/11 : 5, which represents the total of a randomly breeding population, if there is no selection. The condition that the four allelic potencies have the relative magnitudes assumed for *C. fumiferana* in figure 3 is essential; altered relative potencies change the dominance or epistatic relationships and thus lead to different sets of segregation ratios, as will be shown later.

In the top part of table 6 the expected phenotypic frequencies have been calculated assuming that the frequency of the autosomal B(4) gene—the bracketed numeral stands for the potency—ranges from 0 to 1.0, i.e. from its absence to its complete fixation, assuming at the same time an unchanged frequency of .5 for the two sex-linked suppressor alleles. The bottom part of the table gives the same tabulation for the Su(5)-B gene, keeping here the autosomal alleles constant at a frequency of .5. These data are plotted in figure 6, with the superimposed circles representing the approximative phenotypic frequencies of the natural populations. The western two-year cycle population is located near the cross point of the two lines, where the gene frequency is .5 for both loci; the eastern subspecies appears to have undergone a frequency shift at the autosomal locus to .7 for the B(4) gene. This point is indicated in table 6 and the actual observations are in good agreement with the

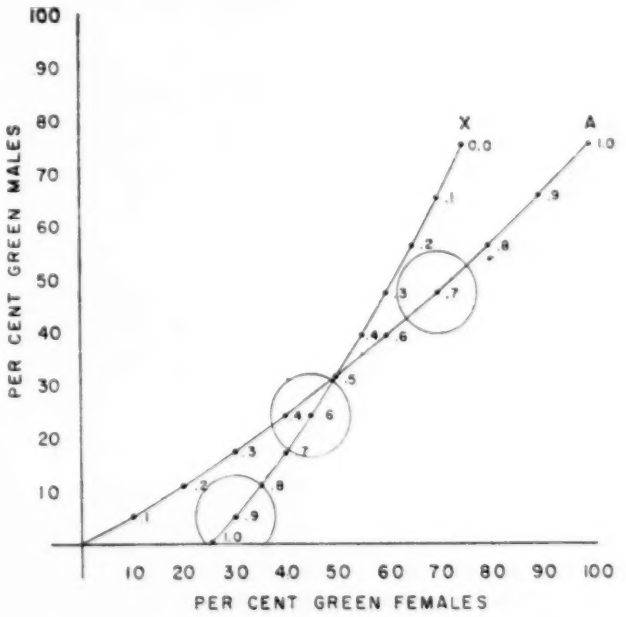


FIG. 6. Relation between various gene frequencies (X, A) and the phenotypic frequencies (abscissa, ordinate) of the sex-controlled hemolymph polymorphism in *C. fumiferana.*

expectation. The western one-year cycle populations show a frequency shift at the sex-linked locus, reaching values of .9 for the Su(5)-B gene close to fixation.

It is of interest to consider the four end constellations of the two lines. In a population at the bottom of the autosomal line, where the B(4) allele is completely replaced by the b(1) allele, all individuals of both sexes would be yellow; at the top, where the b(1) allele is replaced by the B(4) allele, all females would be green, while a quarter of the males would be yellow. Upon finding such a population in nature, this would be described as a polymorphism, sex-limited to males. The genetic basis of this polymorphism would appear in breeding tests to be a "simple" recessive, where the 1 : 1 segregations in F_2, instead of 3 : 1, would prove that the responsible gene is sex-linked, being transmitted through "carrier" daughters. We know, however, that the genotype of the yellow males requires homozygosity

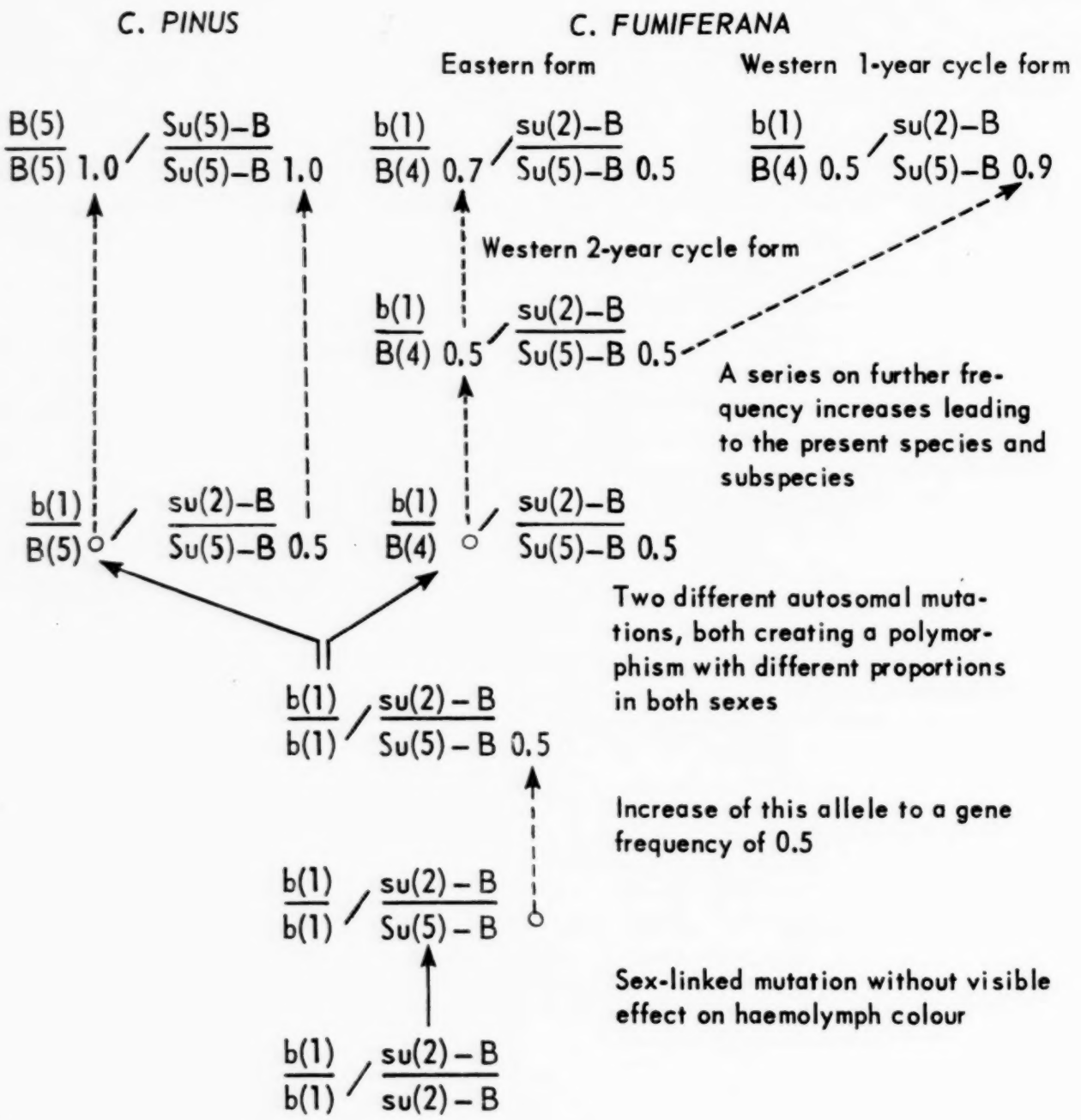


FIG. 7. The hypothetical genetic events that may have differentiated the *C. fumiferana* complex and *C. pinus* with regard to hemolymph polymorphism. In brackets: relative gene potencies, decimal fractions: gene frequencies.

of the suppressor Su(5)-B—thus causing it to be labelled "recessive"—because the autosomal locus always is homozygous for B(4).

In a population at the top end of the frequency range of the X-chromosomal suppressor, with only the su(2)-B allele present, a quarter of both, females and males, are yellow, making for a polymorphism seemingly not controlled by sex. Again a "simple" recessive, and autosomal gene, b(1) would seem to be responsible. At the bottom end, where the Su(5)-B allele would be fixed, all males are yellow and only a quarter of the females green. This then is the very frequently found situation of a polymorphism limited to females. In this theoretical population, closely resembling our western one-year cycle budworm, the polymorphism would appear as being caused by a "simple" recessive autosomal gene, because F_2 females segregate 3 : 1. Actually, B(4) outweighs only in double dose the antagonistic Su(5)-B gene of females, thus causing the green phenotype.

We shall return later to this situation of a polymorphism sex-limited to females, so common in Lepidoptera, and discuss the consequences of some other potency combinations in a system of four alleles on two espistatic loci, one of them sex-linked. At the moment, it appears possible to summarize our present knowledge of the genetic relationships between the different budworm species and subspecies.

Starting with an assumed monomorphic yellow species and using only alleles that are available in the existing natural populations this theoretical population might have had the constitution shown at the bottom of figure 7. In such a population a mutation at the suppressor locus from a potency of 2 to 5 will have no visible effect on hemolymph color, but it sets the stage for a polymorphism of the type found in the *fumiferana* group, as well as providing the proper allele from which the dimorphism of *C. pinus* may have arisen. Only two further mutations need to be assumed, this time at the autosomal locus, creating the B(4) allele in *C. fumiferana* and the B(5) in *C. pinus;* whether these have occurred independently or in stepwise fashion cannot, of course, be decided. After this, various increases in the frequency of these new alleles are all that is required to arrive at the present genetic constitution of the various groups, as revealed in this investigation. It may be emphasized that this scheme does not necessarily trace the actual evolutionary events, which may have been far more involved. It is merely a hypothesis that places the known facts into a consistent framework, making only the fewest and least onerous assumptions.

General Discussion

The taxonomic separation of *C. pinus* from *C. fumiferana* (Freeman, 1953) is fully supported in this study as *C. pinus* does not possess the two alleles of lower potency at the two antagonistic loci and is therefore sex-dimorphic but not polymorphic. Furthermore, it carries on its autosomal protagonistic locus an allele of a higher potency than could be found in *C. fumiferana.* The western forms of the budworm are certainly more closely related to the eastern *C. fumiferana* than to *C. pinus;* they still share with the eastern form all the alleles involved in the blue pigment polymorphism. However, different evolutionary developments have taken place in the east and in the west concerning the gene frequency at the sex-linked antagonistic and the autosomal protagonistic loci (see fig. 6). Should it be possible to confirm the indications of some hybrid inviability in certain subspecific crosses, the question of the taxonomic separation of eastern and western forms may have to be broached. The allelic frequencies are such that the two-year cycle type might be considered as a hybrid between the eastern and the western one-year cycle forms. The unusual generation cycle could then possibly be conceived as a developmental retardation effect on such hybrids, which happily found a suitable ecological niche in the

higher ranges of the Rocky Mountains. On the other hand, our results conclusively exclude the possibility that any of the subspecific groups of *C. fumiferana* could be the outcome of hybridization involving *C. pinus,* as had been suspected by Freeman (1958).

One cannot fail to notice that the genetic basis of the polymorphism in *C. fumiferana* is exactly of a nature that is required of sex differences in general, and is indeed responsible for the sexual dimorphism in *C. pinus.* Goldschmidt (1955, p. 425) summarizes the genetic requirements of sex characters as follows (in part): 1) The existence of a quantitative relation between two sex determiners decides sexuality, that is femaleness, maleness or any grade of intersexuality. 2) One of the two types of sex determiners is located within the X-chromosomes, the other one, outside of them. 3) As a consequence of this the same determiners of a sex character are faced by either one or two portions of those in the X-chromosomes. 4) The balance system works so that two doses in the X-chromosomes are epistatic to the determiners outside the X, but one dose is hypostatic. 5) The action of these determiners can be understood in terms of the kinetics of the reactions controlled by the sex determiners.

Where the determination of a character is divided between an autosomal protagonistic, and an X-chromosomal antagonistic gene, the conditions for a sexual dimorphism are given on the basis of the X-chromosomal/autosomal potency balance, in perfect parallel to the determination of sex as such. However, if there exist, as in *C. fumiferana,* alternative alleles of different relative potencies on one or the other of these loci, many forms of sex-controlled polymorphism and even some that seem not to be sex-controlled, may be created. Such polymorphism thus becomes a phenomenon akin to intersexuality.

Here is not the place for a complete review of the numerous instances of sex-controlled polymorphism. But the great variety of situations, all of which can be covered by the scheme developed here, will have to be exemplified in order to make the hypothesis generally applicable.

In the preceding section we have discussed four theoretical populations assuming gene fixations for each of the possible allelic potencies encompassed by the *fumiferana* polymorphism (1/4; 2/5, mentioning the autosomal locus first). One was devoid of both polymorphism and sex-dimorphism; two involved either female limited or male limited polymorphism; and the fourth a polymorphism seemingly not controlled by sex.

Another potency array, 3/5; 5/8, may now be considered. The simplest description of such a population is given by the two "characteristic segregation ratios" that are obtained by mating the two heterozygous females to the doubly heterozygous male, or by the sum of these, called the "population ratio." In the suggested case, we obtain the population ratio ♀♀ 3 : 5, ♂♂ 1 : 0 that here is identical to the two "characteristic ratios," which, by the way, always differ in males only.

Let us call the first class of the female segregation "normal," as it is identical to that of the males; the second class will be called "alba," because I am going to suggest that the "alba" polymorphism in *Colias* can be correctly interpreted only on the basis of an autosomal/X-chromosomal antagonistic gene pair with relative potencies just mentioned.

Table 7 shows the "alba" and the "normal" genotypes of females and the segregation ratios obtained in matings of these with the nine male genotypes. As may be seen from the data assembled in Remington's review (1954) the interpretation that the "alba" females are due to a dominant autosomal gene meets many difficulties that forced its originator, Gerould, to assume, in turn, lethality of homozygous dominants (1911) and closely linked lethals (1923). The latter assumption was retained by Hovanitz (1944), Ford

TABLE 7. *Interpretation of the sex-limited "alba" polymorphism in* Colias *by an autosomal/X-chromosomal balance system of two antagonistic loci*
Relative potencies of 5 and 3 for the autosomal protagonistic alba alleles A/a, and potencies of 8 and 5 for the sex-linked suppressor alleles Su–A and su–A are assumed. Segregation in females only (top ratio), with the phenotypic alba class given in second place.

Genotypes ♂ / ♀		$\frac{A(5)}{a(3)}/\frac{su(5)-A}{Su(8)-A}$	$\frac{a(3)}{a(3)}/\frac{su(5)-A}{Su(8)-A}$	$\frac{A(5)}{A(5)}/\frac{su(5)-A}{Su(8)-A}$	$\frac{a(3)}{A(5)}/\frac{su(5)-A}{su(5)-A}$	$\frac{a(3)}{A(5)}/\frac{Su(8)-A}{Su(8)-A}$	$\frac{a(3)}{a(3)}/\frac{su(5)-A}{su(5)-A}$	$\frac{a(3)}{a(3)}/\frac{Su(8)-A}{Su(8)-A}$	$\frac{A(5)}{A(5)}/\frac{su(5)-A}{su(5)-A}$	$\frac{A(5)}{A(5)}/\frac{Su(8)-A}{Su(8)-A}$
	Gametic potencies ♂ / ♀	3–5 3–8 5–5 5–8	3–5 3–8	5–5 5–8	3–5 5–5	3–8 5–8	3–5	3–8	5–5	5–8
$\frac{A(5)}{a(3)}/su(5)-A$ "Alba"	3–5									
	5–5	3 : 5	1 : 1	1 : 3	0 : 1	3 : 1	0 : 1	1 : 0	0 : 1	1 : 1
	3	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0
	5									
$\frac{a(3)}{a(3)}/su(5)-A$ "Alba"	3–5	1 : 1	1 : 1	1 : 1	0 : 1	1 : 0	0 : 1	1 : 0	0 : 1	1 : 1
	3	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0
$\frac{A(5)}{A(5)}/su(5)-A$ "Alba"	5–5	1 : 3	1 : 1	0 : 1	0 : 1	1 : 1	0 : 1	1 : 0	0 : 1	1 : 0
	5	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0
$\frac{A(5)}{A(5)}/Su(8)-A$ "Alba"	5–8	1 : 3	1 : 1	0 : 1	0 : 1	1 : 1	0 : 1	1 : 0	0 : 1	1 : 0
	5	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0
$\frac{a(3)}{A(5)}/Su(8)-A$	3–8									
	5–8	3 : 5	1 : 1	1 : 3	0 : 1	3 : 1	0 : 1	1 : 0	0 : 1	0 : 1
	3	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0
	5									
$\frac{a(3)}{a(3)}/Su(8)-A$	3–8	1 : 1	1 : 1	1 : 1	0 : 1	1 : 0	0 : 1	1 : 0	0 : 1	0 : 1
	3	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0	1 : 0

(1953), Komai and Ae (1943), and Remington (1954) among the most recent investigators, and Ford (1957) added the notion of a supergene. All this is attributable to the failure of the data to fit the expected "simple mendelian" 1 : 3 segregations. The failure was in the expectation. The data agree perfectly with a simultaneous occurrence of the various ratios characteristic of a dihybrid balance system of sex-control.

Furthermore, Komai and Ae (1953) and Ae (1957) working on the Japanese *Colias erate* populations give data indicating that some potency mutations occurred there, producing 7 : 1 ratios such as have been found in *C. fumiferana,* and Hovanitz' (1950) data show that gene fixation has taken place in Alaskan populations leading, there, to sex-dimorphism, as in *C. pinus.* The occurrence of occasional white males in *Colias* populations demonstrates also the existence of rare alleles with potency values so extreme that even in the homogametic males the balance may be tipped in favor of the "alba" phenotype.

Figure 8 shows how, within such balance systems mutations that merely change the potencies of an allele—either sex-linked or autosomal—may allow a species to pass from one monomorphic phase through an "autosomal" or "sex-linked" polymorphism, which may or may not be limited to either male or female sex, to a "simple" sex-dimorphism, and from there, again via polymorphism finally to the alternative monomorphic phase. As such polymorphism is thus dependent upon the existence of more than one potency level for either the protagonistic or antagonistic or for both members of the balance system, it becomes understandable that polymorphism frequently is sex-controlled or sex-limited. This is because the chromosomal mechanism of sex-determination provides already two threshold levels thus increasing the chances for a new potency to penetrate one of these, especially the lower one, in the heterogametic sex. The preponderance of female-limited polymorphism in Lepidoptera which intrigued already Goldschmidt (1945), is therefore a consequence of the mechanics of antagonistic gene balance systems.

The conclusion cannot be avoided that the prevailing interpretations of many, if not all, cases of sex-controlled polymorphism are obsolete and deserve reconsideration. This is true of the classical valezina-paphia case, studied by Goldschmidt and Fischer (1922), that led to difficulties similar to those encountered in *Colias,* as well as of the numerous investigations into the genetics of mimetism in

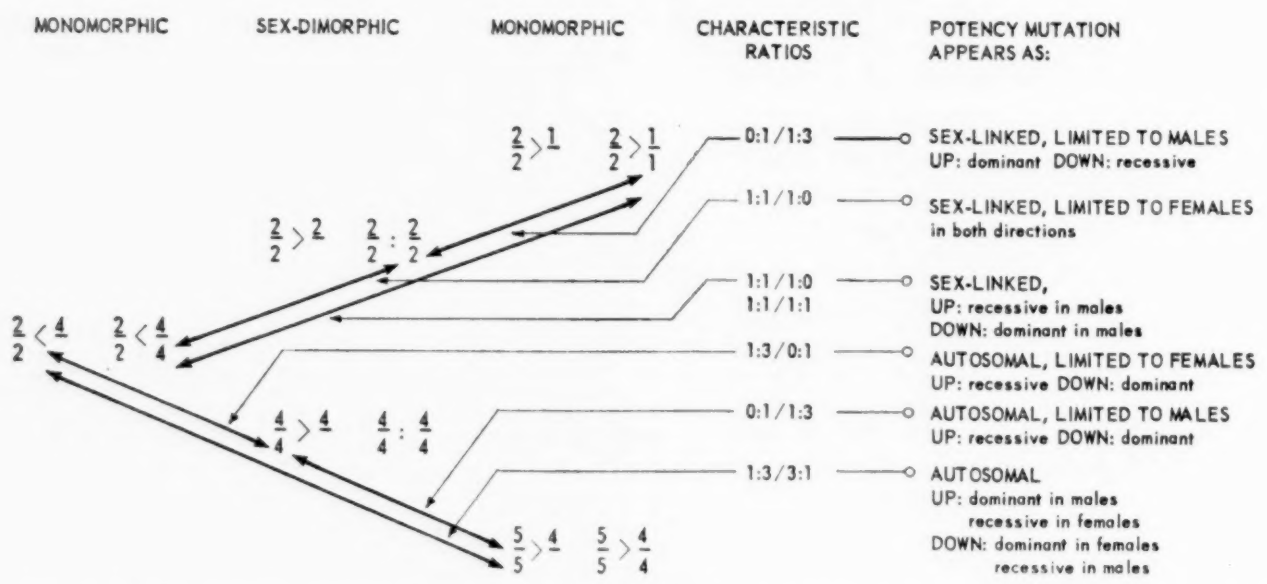


FIG. 8. Some genetic consequences of potency mutations within a balance system of two loci of which one is sex-linked.

butterflies. Clarke and Sheppard (1955), in their study of the genetics of *Papilio machaon,* show clearly that a sex-dimorphism of wing pattern can be obtained in this normally monomorphic species merely by introducing a sufficiently potent allele for the production of black ground-color, and they recognize the possibility that a single locus, which, of course, would have to be a sex-linked inhibitor, might control the differential extension of black ground-color. In his analysis of the mimetism in *Papilio dardanus* Ford (1937) provided evidence for the occurrence of 7 : 1 and 5 : 3 ratios and thus for a balance system of sex-control based on two antagonistic genes.

Actually, Bateson's pupil Fryer (1913) had already interpreted the polymorphism in *Papilio polytes* on the basis of an autosomal gene and an X-chromosomal antagonist ("inhibitor"), thus coming closer to the truth than later investigators. Fryer, however, had no proof—either from heterozygosity or from interracial or interspecific crosses—that the sex-limitation of his characters was actually due to a specific sex-linked antagonistic locus. His inhibitor symbolized the sex-difference in the framework of the presence and absence theory and was not conceived as part of a specific balance system of phenotype determination. When Goldschmidt developed the balance theory of sex-determination, the physiological background difference was considered to be sufficient and sex-controlled characters could be interpreted by their autosomal components only, rendering Fryer's inhibitor locus superfluous (Goldschmidt, 1945).

Goldschmidt (1955) formulated his views—which in this respect are shared generally—in the following manner (p. 427): ". . . any genetically controlled character may have an alternative norm of reaction which is decided by the real primary sex-determining mechanism," and further: ". . . wing color in both sexes is determined by the same genetic determiners, which act with an alternative norm of reaction according to the presence of the sex-determining stuffs of one or the other sex." And finally, sex-controlled inheritance ". . . requires a genuine balance system of sex-determination plus an alternative norm of reaction."

Clearly, sex-controlled inheritance is understood by Goldschmidt in a very literal sense as actually only monogenic inheritance, with phenotypic expression merely modified by the physiologically different background of the sexes. But the specificity of an antagonistic locus on the X-chromosome is now established for the pupal bile-pigment polymorphism of *C. fumiferana,* and the successful interpretation of other long unsettled cases of sex-controlled polymorphism perhaps demonstrates the general validity of the balance mechanism of phenotypic determination. Without recourse, then, to alternative norms of reaction due to physiological or genetical "backgrounds," sex-controlled inheritance is the inheritance of a character that is determined by the very kind of "genuine balance system" that underlies sex-determination itself and indeed is sex-linked in the same way. The mechanisms that determine sex, sex-dimorphisms (or "secondary sex-characters"), and sex-controlled characters are, then, all of the same basic nature: a quantitative relation between two determiners decides alternative phenotypes and one of the two determiners is located on the X-chromosomes, the other outside of them.

Phenotypes, of course, need not be alternatives, but may be a stepwise series of quantitative differences, e.g. as has been shown by Goldschmidt for the melanism in the nun moth (1920). The phenotype may then be an expression of the net surplus potency of the determiners of one or the other kind, and it is symptomatic that similar genotypes of the two sexes show a regular quantitative sequence, as in the nun moth, with the male always one grade darker than the female. Characters so far thought to be determined by multiple genes or polygenes because they appear in graduated series,

as was suggested e.g. by Harvey (1957) for the non-diapause behavior in *Choristoneura,* could be interpreted on a similar basis. The existence of nine genotypes in males and six in females, each with its own net balance and balance level, provides furthermore a more flexible basis for heterosis to protect the simultaneous co-existence of several alleles against selective decimation in cases of balanced polymorphism (Stehr, 1956).

In a postscript, Goldschmidt (Goldschmidt and Piternick, 1957) discusses Glass' (1957) work on the mutant-suppressor system of "erupt" in *Drosophila.* He agrees with him that such systems provide a buffering mechanism that allows for a considerable amount of allelic potency variation without inevitably risking the ill effects of immediate or total phenotypic penetrance, and this without the help of multiple modifiers from the genetic "background." Indeed, as flexibility is a built-in feature of such systems, they possess not only stability but modifiability, and this has a bearing also on the problem of dominance modification. With the provision of several potency levels on both sides of the balance system, the raw material for the selection of the most suitable dominance relationship is available without recourse to multiple dominance modifiers in the "background." This question is important for the theory of mimetism, as is the further point noted by Goldschmidt, that there may be antagonists which are capable of releasing by mutation not only one but a number of protagonistic loci at once.

The phenomenon of dominance clearly testifies to the general pertinence of our view, as it describes nothing other than the size of potency change a mutation must bring about in order to penetrate the antagonistic threshold level; at the same time this means that dominance and epistasy become one and the same, as the potency of the threshold is determined outside the mutant locus. The generally accepted difference between dominance and epistasy, as gene interactions within and between loci, is therefore obsolete and the two are more correctly merged into one. Phenotype is determined dialectically (in the Hegelian sense: synthesis of thesis and antithesis) by the relative balance between the potencies of antagonistic determinants.

The pursuit of this hypothesis may finally open the way for establishing the unity of chromosome function. There may yet be a common bond that gives meaning to one kind of genes being held together here, another kind there. This common bond, is, then, not to be derived from common features of the phenotypic characters but from features common to one series of protagonists, antagonists, or both, which bring about phenotypic determination by entering into a dialectic synthesis with opposite determinants belonging to other such series.

Summary

A sex-controlled polymorphism of pupal hemolymph coloration in the spruce budworm and the sex-dimorphism in the jack-pine budworm are analyzed and both are found to be determined by the relative potency balance between the alleles at a protagonistic autosomal locus and those of an X-chromosomal suppressor locus. Where the total potencies of the autosomal alleles are larger than those of the sex-linked ones, hemolymph is green; if the potencies of the sex-linked alleles are equal or larger, hemolymph is yellow.

Different populations of the spruce budworm show this polymorphism with different phenotypic frequencies. This can be explained by different frequencies for alleles of certain potencies at one or the other of the two antagonistic loci. On this basis a scheme for the possible evolutionary relationship between the budworm species and their subspecific groups has been constructed.

The dialectic modus of phenotype determination by an antagonistic balance system between two loci is parallel to the mechanism of sex-determination as such

and is equally applicable to cases of sex-dimorphic inheritance, sex-controlled or sex-limited inheritance, regardless of the sex involved, as well as to sex-linked inheritance. As an example of its capacity, the "alba" polymorphism in *Colias,* limited to females, has been reinterpreted, thereby eliminating the difficulties that were encountered in previous interpretations.

Literature Cited

Ae, S. A. 1957. Corrections and additions concerning the genetics of Colias erate poliographus (Pieridae). Lepid. News, **10**(5): 143–149.

Campbell, I. M. 1953. Morphological differences between the pupae and the egg clusters of *Choristoneura fumiferana* (Clem.) and *C. pinus* Free (Lepidoptera: Tortricidae. Can. Ent., **85**(4): 134–135.

Clarke, C. A. and P. M. Sheppard. 1955. A preliminary report on the genetics of the *machaon* group of Swallowtail butterflies. Evolution, **9**: 182–201.

Faure, J. C. 1943(a). The phases of the lesser army worm *Laphygma exigua* (Hübn.). Farming in South Africa, 18: 69–78.

——. 1943(b). Phase variation in the army worm, *Laphygma exempta* (Walk.). Dept. Agric. and For. Science Bulletin No. 234: 17 pp.

Ford, E. B. 1937. Problems of heredity in the Lepidoptera. Biol. Rev., **12**: 461–503.

——. 1953. The genetics of polymorphism in Lepidoptera. Adv. in Genetics, **5**: 43–87.

——. 1957. Polymorphism in plants, animals and man. Nature, **180**: 1315–1319.

Freeman, T. N. 1953. The spruce budworm, *Choristoneura fumiferana* (Clem.) and an allied new species on pine (Lepidoptera: Tortricidae). Can. Ent., **85**: 121–127.

——. 1958. The Archipinae of North America (Lepidoptera: Tortricidae). Can. Ent., **90**: (Supp. 7): 89 pp.

Fryer, J. C. 1913. An investigation by pedigree breeding into the polymorphism of *Papilio polytes* Linn. Phil. Trans. of the Roy. Soc., **204**(B): 227–254.

Gerould, J. H. 1911. The inheritance of polymorphism and sex in *Colias philodice.* Am. Nat., **45**: 257–283.

——. 1921. Blue-green caterpillars: the origin and ecology of a mutation in hemolymph color in *Colias* (*Eurymus*) *philodice.* J. Exp. Zool., **34**: 385–415.

——. 1923. Inheritance of white wing color a sex-limited (sex-controlled) variation in yellow pierid butterflies. Genetics, **8**: 495–551.

——. 1926. Inheritance of Olive-green and Blue-green, variations appearing in the life-cycle of a butterfly, *Colias philodice.* J. Exp. Zool., **43**: 413–427.

Geyer, K. 1913. Untersuchungen über die chemische Zusammensetzung der Insekten-hämolymphe und ihre Bedeutung für die geschlechtliche Differenzierung. Z. wiss. Zool., **105**: 349–499.

Glass, B. 1957. In pursuit of a gene. Science, **126**: 683–689.

Goldschmidt, R. 1920. Erblichkeitsstudien an Schmetterlingen III. Der Melanismus der Nonne, *Lymantria monacha* L. Z. ind. Abst. Vererb., **24**: 89–163.

——, and E. Fischer. 1922. *Argynnis paphia-valesina,* ein Fall geschlechtskontrollierter Vererbung. Genetica, **4**: 247–278.

——. 1945. Mimetic polymorphism, a controversial chapter of Darwinism. Quart. Rev. Biol., **20**: 147–164, 205–230.

——. 1955. Theoretical Genetics. University of California Press, Berkeley and Los Angeles.

——, and L. K. Piternick. 1957. The genetic background of chemically induced phenocopies in *Drosophila.* II. J. Exp. Zool., **136**: 201–228.

Goodwin, T. W., and S. Srisukh. 1951. The green pigment of the haemolymph and integument of solitary locusts (*Locusta migratoria migratorioides* R. and F., and *Schistocerca gregaria* Forsk.). Biochem. J., **48**: 199–203.

——. 1952. The biochemistry of locust pigmentation. Biol. Rev., **27**: 439–460 + 2.

——. 1953. The pigments in color phases of the larvae of *Plusia gamma* L. (the Silver-Y Moth). Biochem. J., **55**: 834–838.

Gray, C. H. 1953. The Bile Pigments. Methuen's Monographs on Biochemical Subjects, London.

Gray, P. H. 1953. A note on colors of pupae of *Pieris rapae* developed under artificial conditions. Lepid. News, **7**: 5–6.

Harvey, G. T. 1957. The occurrence and nature of diapause-free development in the spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. J. Zool., **35**: 549–572.

Hidaka, T. 1956. Recherches sur le déterminisme hormonal de la coloration pupale chez Lepidoptères. I. Les effets de la ligature, de l'ablation des ganglions et de l'incision des nerfs chez prépupes et larves agées de quelques Papilionides. Annot. Zool. Japon., **29**: 69–74.

Hovanitz, W. 1944. Genetic data on the two races of *Colias chrysotheme* in North America and on a white form occurring in each. Genetics, **29**: 1–31.

——. 1950. The biology of *Colias* butterflies. II. Parallel geographical variation of dimor-

phic color phases in North American species. Wasmann. J. Biol., **8**: 197–219.

JUNGE, H. 1941. Über grüne Insektenfarbstoffe. Hoppe-Seyler's Z. phys. Chem., **268**: 179–186.

KOMAI, T., AND A. S. AE. 1953. Genetic studies of the Pierid butterfly *Colias hyale poliographus*. Genetics, **38**: 65–72.

MUNSELL. 1929–1942. Book of Color. Pocket Ed., Baltimore, Maryland.

NICKERSON, B. 1956. Pigmentation of hoppers of the desert locust (*Schistocerca gregaria* Forskal) in relation to phase coloration. Anti-Locust Research Centre, London, Bulletin 24: 34 pp.

OKAY, S. 1945. Pigmentation of Orthoptera. Nature, **155**: 635.

——. 1953. Formation of green pigment and color changes in Orthoptera. Bull. Ent. Res., **44**: 299–315.

POULTON, E. B. 1894. The experimental proof that the colors of certain lepidopterous larvae are largely due to modified plant pigments derived from food. Proc. Roy. Soc. London, Ser. A, **54**: 417–430.

REMINGTON, C. L. 1954. The genetics of *Colias* (Lepidoptera). Adv. Genetics, **6**: 403–450.

SMITH, S. G. 1944. The reproduction of the nucleus. Sci. Agric., **24**: 491–509.

STEHR, G. 1954. A laboratory method for rearing the Spruce budworm *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Can. Ent., **86**: 423–428.

——. 1955. Brown female—a sex-linked and sex-limited character in the spruce budworm. J. of Heredity, **46**: 263–266.

——. 1956. On problems and methods in the study of a sex-limited dimorphism. Proc. Tenth Int. Cong. Ent., **2**: 807–809.

NOTES AND COMMENTS

THE GOODNESS-OF-FIT TEST FOR DETECTING NATURAL SELECTION IN RANDOM MATING POPULATIONS

R. C. Lewontin and C. Clark Cockerham

Departments of Genetics and Experimental Statistics, North Carolina State College, Raleigh, N. C.

The issue has recently been raised in the pages of this journal and elsewhere as to the possibility of judging the relative fitnesses of genotypes from the frequencies of these genotypes in populations. Wallace (1958) has provided several numerical examples where severe selection pressures do not result in appreciable deviations of the observed zygotic frequencies from expectation under Hardy-Weinberg equilibrium. Novitski and Dempster (1958) attempted by means of a digital computer to estimate the adaptive values of genotypes in *Drosophila melanogaster* using only the observed genotypic frequencies. They found that the computer produced an infinite variety of best fit values for the fitnesses. We are indebted to them for first calling our attention to this problem and to Dr. Bruce Wallace for urging us to publish our findings.

If the frequency of an allele B in a population is p and that of its alternate allele b ia $q = 1 - p$, then following random mating but *before* any natural selection has occurred the zygotes will be in the relative frequencies

	BB	Bb	bb
(1)	p^2	$2pq$	q^2

Assume now that natural selection operates in such a way that the three genotypes have the fitnesses W_1, W_2, and W_3. Then after selection the genotypes will be in the relative frequencies

	BB	Bb	bb
(2)	$\frac{W_1p^2}{\overline{W}}$	$\frac{2W_2pq}{\overline{W}}$	$\frac{W_3q^2}{\overline{W}}$

where $\overline{W} = W_1p^2 + 2W_2pq + W_2p^2$. The frequency of B is no longer p but p′ where

$$p' = \frac{W_1p^2 + W_2pq}{W_1p^2 + 2W_2pq + W_3q^2}. \tag{3}$$

Suppose, now, that a sample is taken from such a population and that the three genotypes are distinguishable. There will be three observed frequencies, n_1, n_2 and n_3, but only two *independent* observations since there is the linear restriction

$$n_1 + n_2 + n_3 = N_{\text{TOTAL}}.$$

There are, however, four independent parameters, W_1, W_2, W_3 and p. Since the geneticist is usually not interested in the absolute values of the W's but only their relative magnitudes (partly because he does not know how to measure their absolute values), it is possible to reduce the number of independent parameters to be estimated to three, by making W_2 (or W_1 or W_3) equal to unity. However, there is still one more parameter to be estimated than there are available observations, clearly an impossibility.

If estimation of W_1 and W_3 is impossible, is it nevertheless possible to test some hypothesis about these two selection coefficients, or at least to make some judgment about their relative sizes from the observations? Specifically, the geneticist would like to test the hypothesis that $W_1 = W_3 = 1$, that is, that no natural selection is operating. Moreover, if such a hypothesis is rejected, he would, at least, like to know whether W_1 and W_3 are greater or less than unity so that some judgment about the order of fitness of the three genotypes can be made.

The standard procedure presently in use by geneticists is to estimate p by the usual method

$$\hat{p} = \frac{2n_1 + n_2}{2N}$$

and then to test the observed frequencies against the binomial proportions given by (1) with $\hat{p}$ and $\hat{q}$ in place of p and q. If the χ^2 with one degree of freedom is significant it is assumed that natural selection is operating (non-random mating having been ruled out by ancillary information). Moreover, the *directions* of the deviations from expectation are used as a basis for judging the type of selection operating. If, for example, there is an excess of the Bb class with a concomitant deficiency of BB and bb classes, it is assumed that heterozygotes have a greater fitness than homozygotes. A non-significant χ^2 is taken as evidence that $W_1 = W_2 = 1$, that is, that no natural selection is taking place.

What this procedure really amounts to is as follows. First, $\hat{p}$ is an estimate of p′ given by (3) and not of p before selection since no independent estimate of that quantity exists. Second, the expectations calculated are of the form

	BB	Bb	bb
(4)	p'^2	$2p'q'$	q'^2

Finally, the χ^2 calculated is a test of whether the sampled distribution (4) is the same as dis-

tribution (2). These distributions are the same if simultaneously

$$(5) \qquad p'^2 = \frac{W_1p^2}{\overline{W}}, \quad p'q' = \frac{W_2pq}{\overline{W}},$$

and

$$q'^2 = \frac{W_3q^2}{\overline{W}}.$$

The solution to these three equations is simply

$$(6) \qquad W_2^2 = W_1W_3.$$

Thus, what is really tested is that the product of the adaptive values of the homozygotes is equal to the square of the heterozygote fitness. This is a much less restrictive condition than the requirement that $W_1 = W_2 = W_3$. Adaptive values like

BB	Bb	bb
2	1	.50

give a theoretically perfect fit so that the χ^2 test will be significant only α of the time where α is the probability level chosen for the test. Of course if χ^2 *is* significant, there is evidence for natural selection, a point that should not be lost sight of.

The second question of importance is whether the direction of deviations in the goodness-of-fit test gives some hint as to the sort of selection operating. Specifically, does an excess of the heterozygous class mean that the heterozygote has a superior fitness?

The deviation of the heterozygotes from expected is an estimate of

$$(7) \qquad \frac{2pqW_2}{\overline{W}} - 2p'q'$$

which may be greater than, equal to, or less than zero. These three conditions correspond respectively to an excess of heterozygotes, no deviation, and a deficiency of heterozygotes. Substitution of (3) into (7) yields the relationships:

$W_1W_3 < W_2^2$: excess of heterozygotes
$W_1W_3 = W_2^2$: expected heterozygotes
$W_1W_3 > W_2^2$: deficiency of heterozygotes

Thus, an excess of heterozygotes will appear *whenever the geometric mean of the fitnesses of the homozygotes is less than the fitness of the heterozygote.* This obviously *includes* the case of heterosis but is *not restricted* to it. For example, a recessive lethal will cause a considerable excess of heterozygotes since W_1W_3 will be zero. Some numerical examples of this fact have been presented by Wallace (1958).

An extremely important qualification of these results is that they apply only to a population in which gene frequency is changing. *If the gene frequencies are at equilibrium in the population,* $p' = p$, *and the deviations from the Hardy-Weinberg equilibrium are perfectly adequate for estimating* W_1 *and* W_2 *independently.*

Our previous discussion may give the impression that any attempt to determine the sort of selection operating in nature by comparing observed with theoretical frequencies is hopeless. This is not entirely ture. A consideration of the power of the χ^2 test show that it is relatively insensitive to some sorts of selection while being much more sensitive to others. Specifically, the test is much more powerful in detecting heterotic situations than cases of partial or complete dominance.

The power of a test is the probability that the null hypothesis will be rejected (that the test will be significant), when the true parameters of the population differ from the hypothesized parameters by a given amount. Clearly if the true parameters differ from the hypothesized ones by a very large amount, the test will have great power, while for small deviations the power will be small. In addition, for any given deviation, the power of the test will increase with increasing sample size. For a χ^2 test, the power is determined from the distribution of "non-central χ^2" using a "non-centrality" parameter, λ. The larger λ is, the greater the power of the test. For the case in hand the parameter of non-centrality turns out to be:

$$\lambda = \left[\frac{p^2q^2(W_1W_3 - 1)^2}{(pW_1 + 1)^2(qW_3 + 1)} \right] N$$

where W_2 has been fixed at unity. As expected, λ will increase not only with increasing sample size, N, but also as the product W_1W_3 deviates more and more from unity. Note that when W_1W_3 equals unity, λ is zero and the power of the test is zero.

To illustrate the relationship between the kinds of selection and the power of the χ^2 test to detect them, table 1 has been calculated. It is assumed in this table that $p = q = \frac{1}{2}$ and a-l though the numerical values change for other values of p, the qualitative results are the same. What the table shows is the size of sample necessary to be 90% or 50% sure of detecting different combinations of adaptive values of the two homozygotes ($W_2 = 1$).

It is assumed that the 5% level will be used as a significance criterion in the χ^2 test. Across the top of the table are possible values of W_1, the fitness of BB, and along the left hand side are the values of W_3, the fitness of bb. In the body of the table there are two numbers corresponding to each combination of W_1 and W_3. The roman (lower) numbers are the minimum sample sizes necessary to be 90% sure of detecting selection when the true values of W_1 and W_3 are those given at the borders of the table. The italic (upper) numbers give the minimum sample size for 50% power and obviously will in every case be smaller than their corresponding 90% values.

TABLE 1. *Sample sizes necessary to be 50% sure (italic numbers) and 90% sure (roman numbers) of detecting selection when the true fitnesses are those given at the borders of the table*

W_3 = fitness of bb	W_1 = fitness of BB										
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
0.0	*4* 11										
0.1	*5* 13	*6* 16									
0.2	*5* 15	*7* 19	*8* 23								
0.3	*7* 18	*8* 23	*11* 29	*13* 36							
0.4	*8* 21	*10* 27	*13* 35	*17* 45	*21* 57						
0.5	*9* 24	*12* 32	*15* 42	*20* 55	*27* 73	*35* 95					
0.6	*10* 27	*13* 37	*18* 50	*25* 67	*33* 91	*45* 124	*61* 168				
0.7	*11* 30	*15* 42	*22* 59	*30* 82	*42* 116	*59* 162	*85* 231	*123* 337			
0.8	*12* 34	*18* 48	*25* 69	*36* 100	*53* 145	*78* 212	*118* 323	*186* 508	*311* 851		
0.9	*14* 38	*20* 56	*30* 81	*44* 120	*66* 182	*103* 282	*168* 459	*293* 801	573 1567	*1387* 3794	
1.0	*15* 42	*23* 63	*35* 95	*53* 145	*84* 229	*138* 378	*246* 673	*493* 1349	*1244* 3405	*5546* 15,175	— —
1.2	*18* 50	*29* 80	*46* 126	*77* 210	*134* 368	*262* 716	*607* 1660	*2099* 5742	*37,646* 102,999	*10,486* 28,691	*1859* 5086
1.4	*22* 61	*36* 99	*61* 168	*111* 304	*224* 614	*553* 1513	*2212* 6053	*159,847* 437,343	*4978* 13,620	*1181* 3233	*553* 151
1.6	*26* 71	*45* 122	*81* 222	*162* 443	*393* 1074	*1460* 3996	*41,544* 113,665	*5210* 14,254	*1072* 2934	*484* 1325	*2893* 790
1.8	*30* 82	*54* 149	*106* 290	*240* 658	*753* 2060	*6776* 18,538	*12,045* 32,956	*1288* 3523	*504* 1378	*283* 773	*188* 515
2.0	*35* 95	*65* 179	*138* 378	*365* 999	*1694* 4634	— —	*2212* 6053	*625* 1709	*311* 851	*195* 534	*138* 378
2.2	*39* 108	*78* 214	*181* 494	*575* 1573	*5353* 14,647	*8850* 24,213	*983* 2690	*443* 1212	*221* 604	*148* 404	*109* 299
2.4	*44* 121	*93* 255	*236* 647	*957* 2619	*54,910* 150,234	*2497* 6833	*587* 1606	*277* 759	*170* 465	*119* 326	*91* 248
2.6	*50* 136	*110* 301	*311* 851	*1738* 4756	*60,980* 166,841	*1245* 3405	*406* 1112	*214* 585	*138* 378	*100* 274	*78* 213
2.8	*56* 152	*129* 354	*412* 1129	*3662* 10,018	*7549* 20,655	*780* 2134	*307* 840	*174* 476	*117* 320	*87* 237	*68* 187
3.0	*61* 168	*152* 416	*553* 1513	*10,386* 28,416	*3011* 8239	*553* 1513	*246* 673	*147* 401	*101* 277	*77* 210	*61* 168
4.0	*96* 263	*323* 884	*3457* 9458	*4057* 11,100	*523* 2431	*216* 591	*125* 343	*86* 234	*64* 176	*51* 140	*43* 117
10.0	*465* 1272	— —	*669* 1831	*196* 537	*101* 276	*65* 179	*48* 130	*37* 102	*31* 84	*26* 71	*23* 63

The table is divided into three sections by heavy lines. The diagonal stepped line running from the lower left hand corner to the right middle margin separates combinations of W_1 and W_3 which give *excesses* of heterozygotes (above the line) from those combinations giving *deficiencies* of heterozygotes (below the line). The upper area is then subdivided by a horizontal line into an upper region where heterosis obtains, and a lower one in which there is no heterosis. It is this second region which is so troublesome from the standpoint of interpretation of the observations. The worst situation occurs in the three cells containing dashes because here, despite intense selection, there is no observable deviation from expected proportions so that no sample size of whatever magnitude can detect the operation of selection. These are the cases where W_1 is the reciprocal of W_3. The important point about the table is the way in which the sample sizes change with fitness. The upper left hand corner of the table, representing a balanced lethal situation, requires the smallest sample size for detection. Progressing downward and to the right in the table the necessary sample sizes increase until the diagonal is reached, whereupon N begins to decrease. In the case of heterozygote excess it is easier to detect cases of heterosis (above the horizontal line) than those of partial dominance. The χ^2 test is more sensitive to heterotic selection than to other types and is completely incapable of detecting some extreme cases of partial or complete dominance.

It should not be supposed that if the χ^2 test is significant with a sample size of 10, say, that heterosis is demonstrated simply because N = 10 happens to fall above the horizontal line in the table. *Any* type of selection may give a significant χ^2 with *any* sample size. As a matter of fact even when no selection is operating χ^2 will be significant 5% of the time if this is the significance level chosen. All that the table shows is that for a given sample size heterotic situations will be more often detected than non-heterotic ones.

Literature Cited

Novitski, E., and E. R. Dempster. 1958. An analysis of data from laboratory populations of *D. melanogaster*. Genetics, **43**: 470–479.

Wallace, Bruce. 1958. The comparison of observed and calculated zygotic distributions. Evolution, **12**: 113–115.

NOTES ON RELATIVE FITNESS OF GENOTYPES THAT FORMS A GEOMETRIC PROGRESSION.

C. C. Li

Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania

Let the three positive numbers W_1, W_2, W_3 denote the relative selective value (or fitness) of the three genotypes BB, Bb, bb, respectively. In a random mating population the frequencies of the three genotypes are in simple binomial proportions (p^2, $2pq$, q^2) at birth or, more generally, before selection takes place. In the preceding paper Lewontin and Cockerham (1959) have shown that if $W_2^2 = W_1W_3$, the genotypic frequencies *after* the operation of selection in the population will again be in simple binomial proportions (p'^2, $2p'q'$, q'^2) where p' and q' are the new gene frequencies after selection. This fact yields the important conclusion that under such a selective scheme, the observed adult genotype and gene frequencies—presumably those after the operation of selection—will tell us nothing about the value of the W's. The relation $W_2^2 = W_1W_3$ simply means that the three numbers W_1, W_2, W_3 constitute a geometric series. To the author's knowledge, Dempster (1955) is the first who considers such a set of fitness values, taking the W's to be 1, (1-s), $(1-s)^2$ in studying the problem of balance between mutation and selection. The purposes of this note are to supply a model for the genesis of the geometric selective scheme; to point out some simple properties of such a type of selection; and to extend the results to more general cases.

1. First of all, let us review briefly the main result of Lewontin and Cockerham. For their purposes, they find it convenient to make $W_2 = 1$. For our present purpose, however, it is more convenient to take $W_1 = 1$, $W_2 = r$, $W_3 = r^2$, where r may be greater or smaller than unity. When $r = 1$, there is no selection operating. The procedure of analysis and the selection result may be summarized in a tabular form (see table 1). The columns of table 1 are numbered to correspond with the serial number of the expressions in the preceding paper. The only discrepancy is that the common denominator $\overline{W}$ of their expression (2) has been put at the bottom of our column (2). The imposed condition (5) is to

TABLE 1. *Selection in a random mating population when the relative fitness of genotype forms a geometric series.* The relative frequency of genotype after the operation of selection is also in accord with simple binomial proportions.

Genotype	Relative fitness W (6)	Frequency before selection (1)	Frequency after selection (2)	New gene frequency (3)	Calculated genotype frequency (4) = Imposed condition (5)
BB	1	p^2	p^2	$p'=p/(p+qr)$	$p'^2=p^2/\overline{W}$
Bb	r	2pq	2pqr	$q'=qr/(p+qr)$	$2p'q'=2pqr/\overline{W}$
bb	r^2	q^2	q^2r^2		$q'^2=q^2r^2/\overline{W}$
Total		1.0	$\overline{W}=(p+qr)^2$	1.0	1.0 1.0

equate the calculated proportions (4) to the presumably observed relative proportions (2). The solution to satisfy this condition is that the three fitness values form a geometric series (their solution (6)). For the purpose of verification we proceeded with the solution (6) first, that is, to take the fitness values as 1, r, r^2. It is readily seen from our table that in such a case, the relative frequencies in columns (2) and (4) are actually equal.

2. Next, we shall propose a model by which such a set of fitness values may arise. The following model will give the geometric series a simple physical meaning. Most of the statistical techniques in current use deal with linearly additive models. Thus, in quantitative genetics we talk about the additive "genic effect," a quantity to be assigned to each allele in the statistical model. Now the selective values of genotypes may also be analyzed in terms of basic genic selective values. Suppose that the relative fitness of the alleles B and b are 1 and r, respectively. If these fitness values act additively in diploid genotypes, then the fitness values of BB, Bb, bb will be 2, 1 + r, 2r, which constitute an arithmetic progression. On the other hand, if the allelic fitness values act *multiplicatively*, the three genotypic fitnesses will be 1, r, r^2, forming a geometric series. Thus, we conclude that the geometric genotypic selective values arise from the multiplicative compounding of the genic selective values. It should be emphasized that this model does not mean that there is any real selection operating at the gametic stage. It is a statistical model.

3. The result of table 1 and the genic model may be readily extended to autopolyploids of any degree. For example, in a random mating population of autotetraploids in segregation equilibrium with geometric fitness values:

	BBBB,	BBBb,	BBbb,	Bbbb,	bbbb,
f:	p^4,	$4p^3q$,	$6p^2q^2$	$4pq^3$,	q^4,
W:	1,	r,	r^2,	r^3,	r^4,

it will be found that $\overline{W} = (p + qr)^4$, and the new gene frequencies after selection are $p' = p/(p + qr)$ and $q' = qr/(p + qr)$. But for tetraploids it is necessary to caculate the gametic frequencies, the random union of which produces the next generation. The genotype BBBb produces $\frac{1}{2}$ BB and $\frac{1}{2}$ Bb gametes, while the genotype BBbb produces $\frac{1}{6}$ BB, $\frac{4}{6}$ Bb, $\frac{1}{6}$ bb gametes with random chromosome segregation. It is easily found that the gametic output of the selected population (fW) is: p'^2BB, $2p'q'$ Bb, q'^2 bb. Therefore, the estimation of gene frequency and the calculation of expected genotype frequency will also yield a perfect fit with the observed (presumably after the operation of selection), as in the case of diploids, in spite of the selection that may exist.

4. The concept of the multiplicative compounding of genic selective values also permits immediate generalization to the case of multiple alleles. For a general mathematical write up it is convenient to take the relative fitness of the alleles B_1, B_2, B_3, . . . as r_1, r_2, r_3, . . . , and we shall have occasion to use this notation later. The relative fitness of a genotype (say, B_iB_j) is then the product of the respective allelic fitness values (r_ir_j). But for a simple presentation of the situation for three alleles, we may take $r_1 = 1$, $r_2 = r$, $r_3 = t$. The relative fitness value of the six diploid genotypes is given in the upper left portion of table 2. Note that the fitness of the three genotypes with respect to any two of the three alleles forms a geometric progression; thus, for the genotypes B_2B_2, B_2B_3, B_3B_3, we have the fitness values r^2, rt, t^2, respectively. These three numbers are in geometrical progression, being proportional to 1, (t/r), $(t/r)^2$. The entire body of table 2 gives the fitness of the fifteen tetraploid genotypes with respect to three alleles. Some of the tetrapolid genotypes are not explicitly indicated in the table, but it should be quite obvious to which genotype each cell corresponds. For instance, the cell with fitness r^2t corresponds to genotype $B_1B_2B_2B_3$.

Since the selection results for diploids and tetraploids are essentially the same, we shall only give those for diploids. Let q_i be the frequency of allele B_i with fitness r_i in a random mating population before the operation of selection. The average fitness is $\overline{W} = (\Sigma q_i r_i)^2$, and the new gene frequency after selection is $q_i' = q_i r_i / \Sigma q_i r_i$. The calculated genotypic frequencies on the basis of such gene frequencies are, of course, the same as those observed after selection, viz., $q_i'^2 = q_i^2 r_i^2 / \overline{W}$, and $2q_i' q_j' = 2q_i r_i q_j r_j / \overline{W}$.

5. There are some very simple properties associated with the geometric type of selection. First, we observe that the amount of change in gene frequency per generation as calculated from the actual genotypic selection is the same as that given by genic selection and this is true for diploids and polyploids, with two or multiple alleles. For example, the value of q' and thus Δq for the genotypic selection indicated in table 1 is identical with the case in which the genic selective values are 1 : r. Thus.

$$\Delta q = q' - q = \frac{qr}{p + qr} - q = \frac{pq(r-1)}{p + qr}.$$

If we put $r = 1 - s$, the above expression reduces to

$$\Delta q = -\, spq/(1 - sq)$$

which is the familiar expression for genic selection.

The next point to be observed is that if the mutation rate from B to b is μ and if the new gene frequency p' is used for calculating the amount of new mutant genes, then the balance between mutation and selection is obtained when

$$\frac{spq}{1 - sq} = p'\mu = \frac{p\mu}{1 - sq},$$

that is,

$$q = \mu/s.$$

This solution is exact (Dempster 1955), without assuming q, μ, or s to be small.

6. Finally, we may note that the gene frequency in successive generations of geometric selection is a very simple function of the initial gene frequency and the selection ratio. Let the initial frequency of b be $q^{(0)} \equiv q$ (dropping the superscript for simplicity). It will be found by repeated substitution that after n generations of selection (see Table 3 for numerical illustration):

$$q^{(n)} = \frac{q\, r^n}{p + q\, r^n}.$$

On the other hand, it may be noted that if the genotypic fitness were 1, r^n, r^{2n} to begin with, the gene frequency would assume the above value in the very next generation. Hence the principle: n generations of selection with fitness 1, r, r^2 is

TABLE 2. *The relative fitness of genotypes of diploids and tetraploids.* The relative allelic fitness of B_1, B_2, B_3 are taken to be l, r, t, respectively.

	$B_1B_1B_1B_1$	$B_1B_1B_1B_2$	$B_1B_1B_2B_2$	$B_1B_2B_2B_2$	$B_2B_2B_2B_2$
$B_1B_1B_1B_1$	B_1B_1 1	B_1B_2 r	B_2B_2 r^2	r^3	r^4
$B_1B_1B_1B_3$	B_1B_3 t	B_2B_3 rt	r^2t	r^3t	
$B_1B_1B_3B_3$	B_3B_3 t^2	rt^2	r^2t^2		
$B_1B_3B_3B_3$	t^3	rt^3			
$B_3B_3B_3B_3$	t^4				

TABLE 3. *Numerical illustration of geometric selection with $r = 2$ and initial $q = \frac{1}{5}$.* The column Wf is used to calculate $\overline{W}$; and f is the relative frequency of genotypes. The superscript (n) indicates the frequency for the nth generation of selection.

Genotype	W	$f^{(0)}$	$Wf^{(0)}$	$f^{(1)}$	$Wf^{(1)}$	$f^{(2)}$	$Wf^{(2)}$	$f^{(3)}$	$Wf^{(3)}$	$f^{(4)}$	$Wf^{(4)}$	$f^{(5)}$	$Wf^{(5)}$
BB	1	$\frac{16}{25}$	$\frac{16}{25}$	$\frac{4}{9}$	$\frac{4}{9}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{9}$	$\frac{1}{9}$	$\frac{1}{25}$	$\frac{1}{25}$	$\frac{1}{81}$	$\frac{1}{81}$
Bb	2	$\frac{8}{25}$	$\frac{16}{25}$	$\frac{4}{9}$	$\frac{8}{9}$	$\frac{2}{4}$	$\frac{4}{4}$	$\frac{4}{9}$	$\frac{8}{9}$	$\frac{8}{25}$	$\frac{16}{25}$	$\frac{16}{81}$	$\frac{32}{81}$
bb	4	$\frac{1}{25}$	$\frac{4}{25}$	$\frac{1}{9}$	$\frac{4}{9}$	$\frac{1}{4}$	$\frac{4}{4}$	$\frac{4}{9}$	$\frac{16}{9}$	$\frac{16}{25}$	$\frac{64}{25}$	$\frac{64}{81}$	$\frac{256}{81}$
Total		1	$\frac{36}{25}$	1	$\frac{16}{9}$	1	$\frac{9}{4}$	1	$\frac{25}{9}$	1	$\frac{81}{25}$	1	$\frac{289}{81}$
q		$\frac{1}{5}$		$\frac{1}{3}$		$\frac{1}{2}$		$\frac{2}{3}$		$\frac{4}{5}$		$\frac{8}{9}$	
$\frac{q}{p}$		$\frac{1}{4}$		$\frac{1}{2}$		1		2		4		8	

equivalent to one generation of selection with fitness 1, r^n, r^{2n}. The general expression for $q^{(n)}$ above takes even a simpler form when expressed in terms of the ratio q/p. Solving for r^n yields

$$q^{(n)}/p^{(n)} = (q/p)\, r^n.$$

The average fitness of any generation is $\overline{W}_n = \overline{w}_n{}^2$ where $\overline{w}_n = (p + qr^{n+1})/(p + qr^n)$, remembering $q \equiv q^{(0)}$. All of these properties have been illustrated in table 3.

7. For practical purposes the fitness values of genotypes need not be in exact geometric progression to render the one-generation study of selection effects quite fruitless. Wallace (1956), in his two examples, takes W_1, W_2, W_3 as 1, .6, .2 and .2, .6, 1 to show that the ordinary method of comparing the observed with the calculated genotype frequencies actually gives a wrong picture of the selective force. If he used .36 in place of .2 in his examples, he will find that the observed and calculated have a perfect fit. The conclusion that there is no selection would be obviously erroneous. By and large, the more the selection pattern resembles a geometric type, the more futile is the study of one-generation data. We are indebted to Wallace for bringing out this important feature in practical selection genetics.

LITERATURE CITED

DEMPSTER, E. R. 1955. Maintenance of genetic heterogeneity. Cold Spring Harbor Symp., **20**: 25–32.

LEWONTIN, R. C., AND C. C. COCKERHAM. 1959. The Goodness-of-fit test for detecfing natural selection in random mating populations. EVOLUTION, **13**: 561–564.

WALLACE, B. 1958. The comparison of observed and calculated zygotic distributions. EVOLUTION, **12**: 113–115.

ON THE EVOLUTIONARY IMPORTANCE OF CYTOPLASMIC STERILITY IN MOSQUITOES

ERNST CASPARI AND G. S. WATSON

Wesleyan University, Middletown, Conn., and Princeton University Princeton, N. J.

In mosquitoes, it has been repeatedly described that crosses between local races or subspecies may be fertile if the cross is carried out in one direction, completely sterile if it is carried out in the opposite direction. This phenomenon has been thoroughly investigated by Laven (1957) using local populations of *Culex pipiens*. He particularly investigated two strains from Hamburg (*Ha*) and Oggelshausen (*Og*) in which the cross ♀ *Ha* × ♂ *Og* is fertile, while in the cross ♀ *Og* × ♂ *Ha* the offspring die as embryos. He transferred the *Og* genome by 52 successive generations of outcrosses of (♀ *Ha* × ♂ *Og*) hybrids to *Og* males into *Ha* cytoplasm, and found that the resulting animals as males do not produce viable offspring when crossed to *Og* females. Though other interpretations are not rigidly excluded, Laven suggests as the most likely explanation a cytoplasmic difference between strains *Ha* and *Og* which results in incompatability between the cytoplasm of an *Og* ovum and a cytoplasmic component of *Ha* sperm, leading to death of the embryos.

Considering the evolutionary implications of this situation, Laven points out that in this situation genes may pass from strain *Og* into strain *Ha*, but not in the opposite direction. He suggests that a cytoplasmic cross-sterility of the type described may lead to a partial sexual isolation barrier, aiding in this way in the genetic differentiation of local popultations. The following theoretical considerations will show that this is not necessarily the case, in fact that random mating in a mixed population consisting of organisms with the cytoplasms of *Ha* and *Og* will result in the rapid elimination of the *Og* cytoplasm.

Let the frequencies of the cytoplasmic types of individuals in the population at one generation be:

Og		*Ha*	
♀	♂	♀	♂
$\frac{b}{2}$,	$\frac{b}{2}$,	$\frac{a}{2}$,	$\frac{a}{2}$, $(a + b = 1)$

and assume random mating and equal selective values, except that ♀ *Og* × ♂ *Ha* is sterile. In the next generation the proportions of the four types will be:

Og		*Ha*	
♀	♂	♀	♂
$\frac{b^2}{2(1-ab)}$,	$\frac{b^2}{2(1-ab)}$,	$\frac{a^2+ab}{2(1-ab)}$,	$\frac{a^2+ab}{2(1-ab)}$.

Thus, in one generation, the proportion of *Og* cytoplasm will change from b to $\frac{b^2}{(1 - ab)}$, that is by a factor $\frac{b}{(1 - ab)}$. This factor is less or equal to unity since it may be rewritten as $\frac{1 - a}{1 - ab}$ in which the denominator is clearly never less than the numerator.

Moreover, $\frac{b}{(1 - ab)}$ decreases, as b decreases. This means that *Og* cytoplasm is eliminated at an ever increasing rate, as shown in figure 1.

If, therefore, animals containing the cytoplasms of *Og* and *Ha* are brought together in the same population, the cytoplasm derived from *Og* will be selected against and eliminated. Genes associated with the *Og* strain will in the meantime be transferred into *Ha* cytoplasm, so that in this process a single population with genes from both strains becomes established.

It is worthwhile to examine the gene transfer in more detail. Suppose that the *Og* strain is

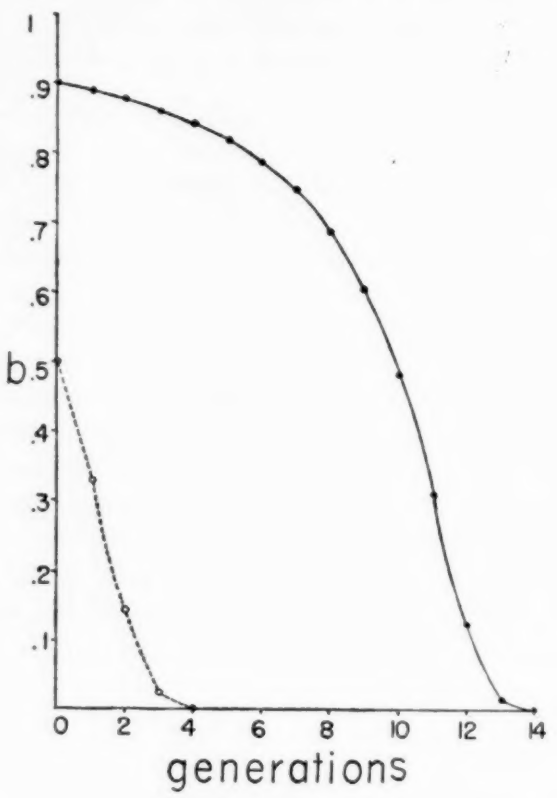


FIG. 1. Values of b (frequency of *Og* cytoplasm in a random breeding population) in successive generations, starting with $b_0 = .9$(•——•) and $b_0 = .5$(o=- - - - -o).

homozygous for a gene c and that the *Ha* strain is homozygous for its allele C. If the initial strain proportions are b_0 and a_0, the gene frequencies would remain b_0 and a_0, were the cross ♀ *Og* × ♂ *Ha* not sterile. Because of the sterility of this cross, the frequency of gene c may ultimately be ***higher*** than it was initially. This may be demonstrated as follows:

Since the cross ♀ *Og* × ♂ *Ha* is sterile, the gene C cannot enter the *Og* cytoplasm so that the population at some general time may be classified as

Og	*Ha*		
cc	cc	cC	CC
b	a_1	a_2	a_3

where the proportions a_1, a_2, a_3 add up to a. The changes in a and b are known from the analysis above. Writing

$$p = \frac{2a_3 + a_2}{2a},$$

= frequency of gene C in *Ha* cytoplasm,

$$q = 1 - p,$$

= frequency of gene c in *Ha* cytoplasm, and,

denoting by $a_1^{(1)}$, $a_2^{(1)}$, $a_3^{(1)}$, $a^{(1)}$, $b^{(1)}$, $p^{(1)}$, $q^{(1)}$ the values of these quantities in the next generation, it is easy to show that

$$a_1^{(1)} = \frac{abq + a^2q^2}{1 - ab},$$

$$a_2^{(1)} = \frac{abp + a^2 2pq}{1 - ab},$$

$$a_3^{(1)} = \frac{a^2p^2}{1 - ab}$$

Since

$$p^{(1)} = \frac{a_2^{(1)} + 2a_3^{(1)}}{2a^{(1)}}$$

we have

$$p^{(1)} = p\,\frac{1 + a}{2}.$$

Since $0 \leq a \leq 1$, $(1 + a)/2$ never exceeds unity and $p^{(1)}$ is never greater than p, i.e., the frequency of the gene C in *Ha* cytoplasm can never increase and will always decrease unless $a = 1$. Initially, $p = p_0 = 1$ and $a = a_0$,

$$p^{(1)} = p_0\,\frac{1 + a_0}{2},$$

$$= \frac{1 + a_0}{2},$$

$$p^{(2)} = \frac{1 + a_0}{2}\,\frac{1 + a_1}{2}, \text{ etc.}$$

In the case $a = 0.1$ as shown in figure 2, after the third generation the frequency of C in *Ha* drops to *less* than its initial value over the whole population.

The final population would have *Ha* cytoplasm and a combination of genes derived from both strains, the frequencies depending on the relative frequencies with which the strains entered the original population.

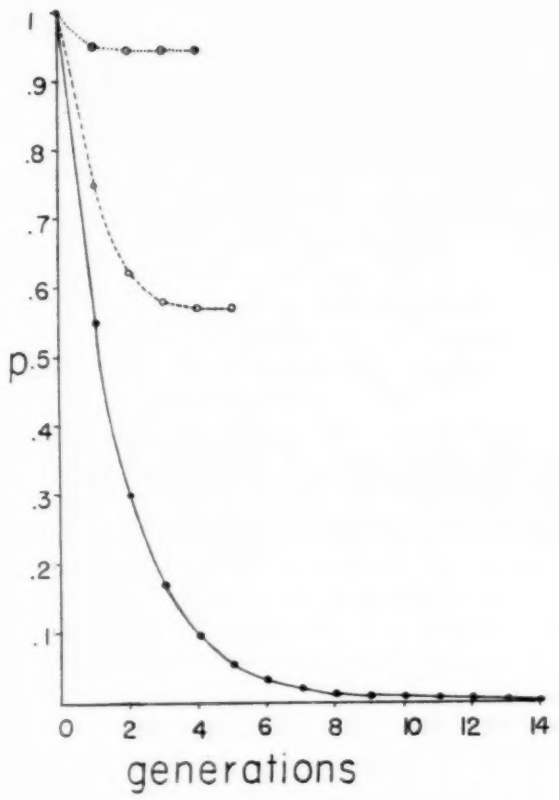


FIG. 2. Values of p (frequency of gene C in *Ha* cytoplasm) in successive generations, if the original frequency of [*Og*] cc in the population is .1(⊙·····⊙), .5(o – – – – – o) and .9(•————•).

If the *Og* cytoplasm has a selective advantage over the cytoplasm *Ha*, the following considerations would obtain: If, as assumed earlier, the adult frequencies of the two cytoplasmic types are

Og		*Ha*	
♀	♂	♀	♂
$\frac{b}{2}$	$\frac{b}{2}$	$\frac{a}{2}$	$\frac{a}{2}$,

and it is assumed that *Og* has a selective advantage $S(S > 1)$ over *Ha*, the adult frequencies in the next generation will be proportional to

Og		*Ha*	
♀	♂	♀	♂
Sb^2	Sb^2	$a^2 + ab$	$a^2 + ab$

Hence

$$b^{(1)} = \frac{Sb^2}{Sb^2 + a^2 + ab}.$$

Thus

$$b^{(1)} \leq b, \text{ if } Sb \leq Sb^2 + a^2 + ab$$

$$\text{i.e. if } (S - 1)b \leq a$$

$$\text{i.e. if } \frac{b}{1 - b} \leq \frac{1}{S - 1}.$$

If therefore initially $\frac{b}{1-b} < \frac{1}{S-1}$, the frequency of *Og* tends to zero, in other words, *Og* cytoplasm would be eliminated.

On the other hand,

$$b^{(1)} \geq b \text{ if } \frac{b}{1-b} = \frac{1-a}{a} = \frac{1}{a} - 1 \geq \frac{1}{S-1}$$

$$\text{i.e. if } \frac{1}{a} \geq 1 + \frac{1}{S-1} = \frac{S}{S-1}$$

$$\text{i.e. if } a \leq \frac{S-1}{S}.$$

Hence, if initially $a < \frac{S-1}{S}$, b will increase and the proportion of *Og* cytoplasm tends to unity.

The equilibrium value is at

$$a = 1 - \frac{1}{S}, \quad b = \frac{1}{S}.$$

This, by the above, is a metastable equilibrium. Either *Ha* or *Og* cytoplasm would be eliminated for frequencies on either side of this point. In the long run, a population of the type described should obtain a uniform cytoplasm.

Literature Cited

LAVEN, H. 1957. Vererbung durch Kerngene und das Problem der ausserkaryotischen Vererbung bei Culex pipiens. II. Ausserkaryotische Vererbung. Z. ind. Abst. Vererbl., **88**: 478–516.

EXCHANGE OF GENETIC MATERIAL[1]

PATRICIA ST. LAWRENCE

Department of Genetics, University of California, Berkeley

"The problem of genetic recombination is broadly interpreted by the organizers of this Symposium, to include processes involved in the synthesis of genetic materials, processes of recombination within gene loci and between closely-linked loci, classical crossing over in Drosophila, consequences of recombination within populations, and the effect of recombinnation on gene stability." These words, quoted from Demerec's foreword, indicate both the intent and extent of the Cold Spring Harbor Symposium held in June, 1958.

The replication and recombination of desoxyribonucleic acid (DNA) is discussed in the initial part of the Symposium. Meselson and Stahl report on the transmission of N^{15} labeled parental DNA in *E. coli* during two division cycles. The DNA of *E. coli* is shown to consist of two molecular sub-units, each one-half the molecular weight of the parental DNA and each transmitted to a daughter cell upon division. The conclusion can therefore be drawn that the replication of DNA in *E. coli* occurs as predicted from the Watson-Crick (1953) model.

Meselson and Stahl discuss the analogous findings of Taylor *et al.* (1957) on the distribution of tritium-labeled chromosomes during mitosis in plants. These authors consider that, at present, a number of plausible chromosome models relating DNA structure to chromosome organization can be made.

One such model of the arrangement of the DNA in the chromosome is described by Freese in the succeeding paper. He points out how his model can account for some aspects of gene and chromosome behavior such as mutation, recombination, and sister strand exchange. These phenomena are probably as readily accounted for on the basis of the Watson-Crick model of DNA alone. For any extension of this model to chromosome structure, two exigent aspects of *chromosome* behavior would seem to demand explanation: the pairing process and positive interference. These do not appear to have been considered in Freese's or in other models (e.g. Taylor, 1957; Schwartz, 1955).

It might appear that genetic exchange in the bacteriophages T2 and T4 would be more readily comprehended than in the compound chromosomes of higher organisms. Yet the trenchant and critical review by Hershey does not inspire confidence that all is completely understood about the mechanics of recombination in phage crosses. In his summary, he evaluates the copy-choice mechanism of recombination (Lederberg, 1955). This hypothesis of recombination continues to have an irresistible appeal because it permits the integration of a variety of phenomena observed in phage, bacteria, and fungi.

Anderson's discussion of his extensive analyses of single zygote pedigrees following mating in *E. coli,* and the similar studies of Luria and

[1] Cold Spring Harbor Symposia on Quantitative Biology. 1958. Vol. 23. xvi + 433 pp. The Biological Laboratory. Cold Spring Harbor.

co-workers on the descendants of Salmonella cells infected with *c* phages, explore the early history of the introduced material (Hfr chromosome fragment, and phage, respectively). In both cases the infective agent may not be fully incorporated into the recipient genome for a time after its introduction. The Hfr material provides a source from which portions may be copied and integrated with the genetic structure of the recipient. Some restriction upon the freedom (or ease) of copying, or of integrating the copy is invoked by Demerec *et al.* to explain their findings of the preferential incorporation of wild-type alleles in recombinants during transduction in Salmonella.

Four papers (by de Serres; Case and Giles; Roman and Jacob; and Leupold) are devoted to a consideration of various aspects of non-reciprocal recombination in fungi. The analysis by Case and Giles of tetrads from crosses between two strains of Neurospora blocked in the same step in the synthesis of pantothenic acid provides the most impressive array of information yet available. Both reciprocal and non-reciprocal recombination were found, and asci showing aberrant ratios of the pantothenate alleles were also obtained.

Case and Giles consider what mechanisms may explain their results and appear to favor a scheme developed by Chase and Doermann (1958) and extended by Freese (1957) to all recombination, both reciprocal and non-reciprocal. Freese assumed that regions of "intimate pairing" are distributed throughout the chromosome; in these regions the newly-arising strands copy from one or the other parent and may switch from one parent to the other.

de Serres also favors the unitary hypothesis of a single mechanism for the origin of reciprocal and non-reciprocal recombination but mentions a serious obstacle. An extensive body of evidence exists suggesting that DNA synthesis is complete in most organisms before the occurrence of crossing over (reviewed by Taylor, 1957).

Roman and Jacob argue for separate mechanisms of reciprocal and non-reciprocal recombination. They report that non-reciprocal recombination in yeast is increased by U.V. irradiation. A similar stimulation of recombination is observed in phage (Jacob and Wollman, 1955) and may be due to the involvement of DNA in both organisms. Roman and Jacob speculate that reciprocal recombination, on the other hand, might be ". . . characteristic of another material, protein for example."

In view of the low signal-to-noise ratio in the debate about the nature of recombination at what is believed to be a molecular level, those participants who grapple with the intricacies of crossing over and interference (Weinstein) and of exchange in the compound X chromosomes of Drosophila (Sandler) seem brave men indeed. Oksala attempts to explain interchromosomal effects on crossing over in *D. melanogaster* in terms of association of the heterochromatic regions. He also discusses factors controlling secondary non-disjunction. This appears to be the only consideration of mechanisms of preferential segregation in the Symposium. It is surprising that the regulation of the distribution of particular chromosomes or of whole sets of chromosomes during meiosis receives so little attention.

In his paper describing the non-random associations of inversions in the metacentric X chromosome of *Drosophila robusta,* Levitan reports that recombination between an inversion in one arm and an inversion in the other has been shown to occur both in the laboratory and in the wild. Nevertheless, the possible combinations of these inversions with each other and with their normal homologues are not recovered with the frequencies expected on the basis of random association.

Several papers relate explorations of the extensive genetic diversity maintained in well-adapted cross-breeding populations of Drosophila. Spiess discusses the effects of recombination on variability in three species of Drosophila in an attempt to determine how much of the variability observed under inbreeding conditions is inherently present but concealed in linked complexes of genes and how much is associated with the disruption of linked complexes as the result of genetic exchange.

In considering the aspects of gene action which underlie his results, Spiess points out the inadequacy of a model requiring additive polygenes to account for the consistent lowering of viabilities he observes for the recombined products of chromosomes of different origin. He suggests that ". . . viability is largely dependent upon either the *sequence* of modifiers or the particular juxtaposition of such modifiers."

It is tempting to speculate that this emphasis upon arrays of genes along the chromosome may be related to the instances of close linkages between genes controlling sequential steps in biosynthesis recently recognized in several microorganisms. Such spatial organization of genes might reflect some evolutionary advantages of proximity to the efficiency of a biosynthetic process. It would not be surprising, therefore, if disruption of a fixed gene order had a qualitative or quantitative effect on gene function and even led to the production of "synthetic lethals." Demerec *et al.* briefly review the findings of proximity in Salmonella, and Austrian *et al.* report on similar linkages in transformation of factors controlling the synthesis of a polysaccharide in Pneumococcus.

Mangelsdorf's interesting report of mutability

brought about by hybridizing maize and teosinte may suggest some additional approaches to an understanding of the effects of recombination in Drosophila populations. The phenomenon of instability of the genotype in interspecific hybrids of Gossypium and Nicotiana is also briefly discussed by Gerstel and Phillips. These are dramatic examples of the effect of removal of genes from their accustomed milieu and their insertion into another cellular environment.

Another type of effect on gene action is described by Brink in maize. This is the invariable occurrence of a change in action of the R^r gene in progeny derived from plants having R^r in heterozygous condition with certain of its alleles. Brink calls this phenomenon *paramutation*. On the basis of his latest results, he speculates that the paramutagenic effect may be associated with a genic component which may be removed from the *R* locus by transposition or recombination.

The effects of different degrees of inversion heterozygosity on selection for quantitative characters are discussed in two papers. Mitchell concludes that multiple inversion heterozygosity in chromosomes 2 and 3 increases the rate of release of variability present in the structurally normal X chromosomes derived from two different inbred lines of *D. melanogaster*. By contrast, Carson shows that selection for motility is most effective in lines permitting the maximum amount of genetic exchange.

In a second group of papers, various restrictions upon the freedom of genetic exchange are discussed by several authors. Lorković considers the importance of spatial and sexual isolation in restricting the gene exchange among allopatric species of the butterfly, Erebia, and Grant reviews the many factors regulating processes of recombination in plants. Stebbins seeks to explain the constancy of genotype imposed by the development of self-fertilization or apomixis among the Compositae in terms of the advantage conferred by such systems in particular environments. White describes the prevalence of inversion heterozygosity in grasshopper populations and the consequent restriction on the free exchange of genetic material among these insects.

Complex and little understood as the interaction of genes in an organism or in a population may be, the relationship between the nucleus and the cytoplasm is even less clear. The three papers (by Srb; Catcheside and Overton; Roper) concerned with nuclear-cytoplasmic relations are of interest in providing new approaches to a consideration of this basic problem.

Catcheside and Overton discuss the observations of complementation between alleles in heterocaryons of Neurospora. These cases constitute evidence not previously available for some recombination in the cytoplasm between the immediate or remote products of genes located in separate nuclei. The mechanism underlying this process awaits discovery.

An analysis of three acriflavin-induced mycelial variants in *Aspergillus nidulans* is presented by Roper. The mycelial phenotype is conditioned by a joint action of a cytoplasmic state with an appropriate genotype. In crosses of mycelial to normal, all the products carry the mycelial determinant, and in heterocaryons between normal and mycelial strains transmission of the mycelial character to the normal nucleus is observed at low frequency. Transmission is interpreted as due either to some mechanism such as transduction or to some cytoplasmic determinant which must, however, be nucleus-bound.

The quasi-nuclear, quasi-cytoplasmic state of the mycelial character is reminiscent of the behavior in bacteria of several determinants designated "episomes" by Wollman *et al.* (1956). It would indeed be of great interest if it could be shown that infective heredity exists in higher organisms. The similarities between the mycelial determinant and the F factor of *E. coli* were pointed out by Luria in discussion.

It is hoped that this review has served to indicate the wealth and diversity of information presented at this Symposium. The volume is not recommended to the beginning graduate student who desires a quick survey of the process of recombination. After a few hours with the report, one no longer understands what recombination is: the word dissolves into a series of problems at different levels of integration. The absence of party lines is at once refreshing and distracting. But certainly the vigor of the attack on all fronts must shortly reveal the secrets of this, after all, every-day phenomenon. Or should it be phenomena?

Literature Cited

Chase, M., and A. H. Doermann. 1958. High negative interference over short segments of the genetic structure of bacteriophage. Genetics, **43**: 332–353.

Freese, E. 1957. The correlation effect for a histidine locus of *Neurospora crassa*. Genetics, **42**: 671–684.

Jacob, F., and E. L. Wollman. 1955. Etude génétique d'un bactériophage tempéré d'*Escherichia coli*. III. Effect du rayonnement ultraviolet sur la recombinaison génétique. Ann. Inst. Pasteur, **88**: 724–749.

Lederberg, J. 1955. Recombination mechanisms in bacteria. J. Cellular Comp. Physiol. (Suppl. 2), **45**: 75–107.

SCHWARTZ, D. 1955. Studies on crossing over in maize and Drosophila. J. Cellular Comp. Physiol. (Suppl. 2), **45**: 171–188.

TAYLOR, J. H. 1957. The time and mode of duplication of chromosomes. Am. Naturalist, **91**: 209–221.

——, P. S. WOODS, AND W. L. HUGHES. 1957. The organization and duplication of chromosomes as revealed by autoradiographic studies using tritium-labeled thymidine. Proc. Natl. Acad. Sci., **43**: 122–128.

WATSON, J. D., AND F. H. C. CRICK. 1953. The structure of DNA. Cold Spring Harbor Symp. Quant. Biol., **18**: 123–131.

WOLLMAN, E. L., F. JACOB, AND W. HAYES. 1956. Conjugation and genetic recombination in *Escherichia coli* K-12. Cold Spring Harbor Symp. Quant. Biol., **21**: 141–162.

FORERUNNERS OF DARWIN

CONWAY ZIRKLE

University of Pennsylvania, Philadelphia

Forerunners of Darwin[1] consists of fifteen essay-chapters contributed individually by seven authors. Six of the essays are by Arthur O. Lovejoy, three are by Bentley Glass, two by Francis C. Haber and one each by Lester C. Crocker, Charles C. Gillispie, Jane Oppenheimer, and Owsei Temkin. Nine of the essays are published here for the first time, six are republished. Five of Lovejoy's, one of Glass' and a portion of Gillispie's have been printed earlier but are revised here and brought up to date. The collection as a whole is an authentic and valuable bit of scholarship that has made a surprising amount of valuable information easily accessible.

In the *Preface,* Glass has stated, "This is the way the book has grown, a somewhat haphazard way governed by individual predilections, and therefore explaining its obvious omissions and lapses. If there be countervailing merit, perchance it lies in the enthusiasms with which the several authors have treated their chosen topics." To this the reviewer would add that any aspects of a very large subject that the book may omit are more than made up for by the depth of the individual essays. As we would expect, any book that contains six chapters by Lovejoy is well worth reading and, when the other authors attain his standards, as they have here, the result is an important contribution to our knowledge. What the work covers, it covers exceptionally well.

For convenience the chapters are arranged in three divisions. The *Introductory Background* describes the status of geology and of biological species at the mid-eighteenth century. The section on *The Eighteenth Century* records the evolutionary contributions of Maupertuis, Buffon, Diderot, Kant and Herder, and discusses in addition the prevailing notions of heredity and variation and the contemporary understanding of fossils. *The Nineteenth Century* evaluates the role of Lamarck and Darwin in the history of science and the evolutionary concepts of Schopenhauer. Such factors of evolution as embryological recapitulation, the contemporary idea of descent are also included here as are some of the pre-Darwinian arguments for evolution.

A number of worthy popular and semi-popular works on evolution are appearing in this the hundredth anniversary of the *Origin of Species. The Forerunners of Darwin,* however, is a book for professionals. Biologists and historians of science will find it to be very convenient to keep a copy of the work where they can refer to it easily.

[1] Glass, Bentley, Owsei Temkin and William Straus, Jr. 1959. Forerunners of Darwin 1745–1859. 471 pp. $6.50. Johns Hopkins University Press. Baltimore.

BOOKS RECEIVED

Notices will be given under this heading of books of evolutionary interest sent to the Editors. Appearance of a title in this section will not preclude a fuller review in a later issue of the journal.

Millhauser, Milton. 1959. Just Before Darwin. ix + 246 pp. 2 plates. $4.50. Wesleyan University Press. A biography of Robert Chambers, author of the famous *Vestiges of the Natural History of Creation,* and a well-

documented study of the impact of the book on the British intellectual climate of the day.

Anfinsen, Christian B. 1959. The Molecular Basis of Evolution. xiii + 228 pp. 99 figs. Price not stated. John Wiley. An examination of the molecular basis of genetics and its evolutionary implications by a biochemist.

Saunders, J. T., and S. M. Manton. 1959. A Manual of Practical Vertebrate Morphology. viii + 272 pp. 55 figs. $7.00. Oxford University Press. The third edition of the textbook of zoology based on the Natural Sciences Honors course at Cambridge University.

Hardin, Garrett. 1959. Nature and Man's Fate. xi + 375 pp. illus. $6.00. Rinehart, N. Y. A lucid account of current evolutionary thought and its development for the intelligent layman.

Roe, Anne and George Gaylord Simpson (editors). 1958. Behavior and Evolution. viii + 557 pp. illus. $10.00. Yale University Press. Proceedings of a symposium under partial sponsorship of the Society for the Study of Evolution. The twenty-three papers deal with the general background of evolutionary studies, the physical basis and categories of behavior, the place of behavior in the study of evolution, evolution and human behavior, and include an introductory and a summarizing essay by one of the editors.

Hill, W. C. Osman. 1959. The Anatomy of *Callimico goeldii* (Thomas). 116 pp. 78 figs. $2.50. Am. Philos. Soc. (Transactions, v. 49, part 5). An assessment of the phylogenetic position of a primitive South American Primate genus on the basis of an approach from detailed comparative anatomy.

ERRATA

At the bottom of the right-hand side of page 339 of the September, 1959, issue several lines are missing. Beginning with the words "It appears . . ." on line 8 from the bottom, the correct text should read:

. . . It appears that the class originated from a primitive Ascomycete akin to the modern genus *Taphrina*. In *Taphrina* the spores are formed within the simple ascus, are liberated when the ascus bursts on being flooded with water and are dispersed in water. Occasionally, but aberrantly, *Taphrina* . . .

The second line from the bottom on the left-hand side of page 378 should read:

"peculiar situation. The whole group of"

TABLE OF CONTENTS

NUMBER 1, MARCH

Polygenes in Eye Pigment Production in Drosophila Species. D. J. NOLTE 1
Late Cenozoic Evolution of the Sierran Bigtree Forest. DANIEL I. AXELROD 9
Polyploidy in Gymnosperms. T. N. KHOSHOO 24
Speciation in Butterflies of the *Papilio glaucus* Group. I. Morphological Relationships and Hybridization. LINCOLN P. BROWER 40
A Study of Hybrids in *Colias* (Lepidoptera, Pieridae). SHIGERU ALBERT AE 64
Geographical Differentiation of Inversion Systems in *Drosophila subobscura*. ELISABETH STUMM-ZOLLINGER and ELISABETH GOLDSCHMIDT 89
The Pelycosaur Tympanum and Early Evolution of the Middle Ear. NICHOLAS HOTTON III 99
Adaptive Shift and Dispersal of a Tropical Ant Fauna. EDWARD O. WILSON 122
Notes and Comments
Variability and Environment. J. HESLOP-HARRISON 145
The Antennae of Drosophila Females. LEE EHRMAN 147
Some Comments on the Evolution of Arthropoda. HOWARD P. EVANS .. 147
Perspectives in Marine Biology. RALPH G. JOHNSON 149
Announcements 150

NUMBER 2, JUNE

A Unique Balanced Breeding System in the Vernal Pool Mouse-Tails. DONALD E. STONE 151
Nasal Anatomy and the Phylogeny of Reptiles. THOMAS S. PARSONS 175
Chromosomal Evolution in the Subtribe Asterinae. YUKIO HUZIWARA 188
Preadaptation and Multiple Evolutionary Pathways. WALTER J. BOCK 194
Speciation in Butterflies of the *Papilio glaucus* Group. II. Ecological Relationships and Interspecific Sexual Behavior. LINCOLN P. BROWER . 212
Evolution in Upper Jurassic Ammonites. BERNHARD ZIEGLER 229
Population Analysis of a Hybrid Swarm between *Gambusia affinis* and *G. heterochir*. CLARK HUBBS 236
Contact between the Ranges of Two Allopatric Species of Pocket Gophers. THOMAS E. KENNERLY, JR. 247
Evolution of the Psilophyte Paleoflora. DANIEL I. AXELROD 264
Notes and Comments
Chromosomal Aberrations in *Podophyllum peltatum*. LESTER J. NEWMAN 276
Is *Hordeum agriocrithon* the Ancestor of Six-Rowe Cultivated Barley? DANIEL ZOHARY 279
"Origins of Angiospermous Plants." VERNE GRANT 281
Books Received 282
Errata 282

NUMBER 3, SEPTEMBER

Multivariate Geographical Variation in the Wolf, *Canis lupus* L. PIERRE JOLICOEUR 283
Barriers to Gene Exchange within *Mimulus guttatus* (Scrophulariaceae). ROBERT K. VICKERY, JR. 300
Natural Triploids in the Orchard Grass, *Dactylis glomerata* L. Polyploid Complex and Their Significance for Gene Flow from Diploid to Tetraploid Levels. DANIEL ZOHARY and UZI NUR 311
Population Studies on *Viola lactea* Sm. and its Wild Hybrids. D. M. MOORE 318
Limited Penetration of Barriers as a Factor in Evolution. D. B. O. SAVILE .. 333
The Evolution of Mammalian Characters. EVERETT C. OLSON 344
Eocene Radiation and Phylogeny of the Rodents. ALBERT E. WOOD 354
Round Trip from Space. MELVIN CALVIN 362
Evolution in the Genus *Geum*. W. GAJEWSKI 378
Genetics of Natural Populations. XXVIII. Supplementary Data on the Chromosomal Polymorphism in *Drosophila willistoni* in its Relation to the Environment. A. BRITO DA CUNHA, TH. DOBZHANSKY, O. PAVLOVSKY, and B. SPASSKY 389
Mesozoic Mammals and the Polyphyletic Origin of Mammals. GEORGE GAYLORD SIMPSON 405
Notes and Comments
The Perianth of *Aristolochia*—a New Interpretation. JACOB LORCH 415
Dating the Origin of Life on Earth. MICHAEL H. BRIGGS 416
Lepturus and *Monerma:* a Remarkable Example of Parallel Development of Gross Morphology in Grasses. TUGUO TATEOKA 418
Comments on Quantitative Systematics. ROBERT R. SOKAL 420
Reply to Sokal. ROBERT F. INGER 423
Books Received 423

NUMBER 4, DECEMBER

Canalization of the Scute Phenotype of Drosophila. J. M. RENDEL 425
Some Aspects of Evolution in Lake Nyasa. GEOFFREY FRYER 440
Call Differentiation in a Complex of Seven Species of *Crinia* (Anura, Leptodactylidae). MURRAY J. LITTLEJOHN 452
Ecotypic Variation in *Nigella arvensis* L. YOAV WAISEL 469
Discrete Populations Derived by Interspecific Hybridization and Selection in *Nicotiana*. HAROLD H. SMITH and KEVIN DALY 476
Area, Climate and Evolution. PHILIP J. DARLINGTON, JR. 488
Delphinium gypsophilum, a Diploid Species of Hybrid Origin. HARLAN LEWIS and CARL EPLING 511
Chromosomal Distribution of Mutator- and Radiation-induced Mutations in *D. melanogaster*. P. T. IVES 526
Ecological and Physiological Observations on Polymorphic Populations of the Toad, *Bufo arenarum* Hensel, from Argentine. J. M. CEI 532
Hemolymph Polymorphism in a Moth and the Nature of Sex-controlled Inheritance. G. STEHR 537

Notes and Comments

The Goodness-of-fit Test for Detecting Natural Selection in Random Mating Populations. R. C. LEWONTIN and C. CLARK COCKERHAM ... 561

Notes on Relative Fitness of Genotypes that Forms a Geometric Progression. C. C. LI ... 564

On the Evolutionary Importance of Cytoplasmic Sterility in Mosquitoes. ERNST CASPARI and G. S. WATSON ... 568

Exchange of Genetic Material. PATRICIA ST. LAWRENCE ... 570

Forerunners of Darwin. CONWAY ZIRKLE ... 573

Books Received ... 573

Errata ... 574

Index for Volume XIII ... vi

INDEX TO VOLUME XIII, 1959

Adaptive shift and dispersal of a tropical ant fauna, 122
Ae, Shigeru Albert, A study of hybrids in *Colias* (Lepidoptera, Pieridae), 64
Allopatric species of pocket gophers, 247
Ammonites, upper Jurassic, 229
Amphidiploids in *Nicotiana,* 476
Anatomy of *Callimico goeldii* (Thomas), The, (notice of book), 574
Anfinsen, Christian B., The Molecular Basis of Evolution, (notice of book), 574
Announcements:
  Karl P. Schmidt Fund, 150;
  National Science Foundation, 150
Antennae of Drosophila, removal of, 147
Ants, tropical, adaptive shift and dispersal in, 122
Anura, mating calls, 452; hypnotic reflex in, 532
Area, climate and evolution, 488
Aristolochia perianth, 415
Arthropoda, evolution of, 147
Asterinae, chromosomal evolution in, 188
Axelrod, Daniel I., Evolution of the psilophyte paleoflora, 264; Late Cenozoic evolution of the Sierran Bigtree forest, 9
Balanced breeding system in vernal pool mouse-tails, 151
Barley, *H. agriocrithon* as ancestor of, 279
Barriers, geographical and ecological, in evolution, 333; correction, 574
Barriers to gene exchange in Mimulus guttatus, 300
Basitemporal articulation, avian, 194
Behavior and Evolution, (notice of book), 574
Bigtree forest evolution, 9
Bock, Walter J., Preadaptation and multiple evolutionary pathways, 194
Books, notices and reviews:
  Anfiinsen, Christian B., The Molecular Basis of Evolution, 574
  Buzzati-Traverso, A. A. (editor), Perspectives in Marine Biology, 149
  Cold Spring Harbor Symposium on Quantitative Biology, vol. XXIII, 282, 570
  Glass, Bentley, Owsei Temkin, William L. Straus, Jr. (editors), Forerunners of Darwin: 1745–1859, 423, 573
  Hill, W. C. Osman, The Anatomy of *Callimico goeldii* (Thomas), 574
  Millhauser, Milton, Just before Darwin, 573
  Padoa, Emanuele, Storia della Vita sulla Terra, 282
  Roe, Anne and George Gaylord Simpson (editors), Behavior and Evolution, 574
  Romer, A. S., The Vertebrate Story, 282
  Saunders, J. T. and S. M. Manton, A Manual of Practical Vertebrate Morphology, 574
  Takhtajian, A. L., Origins of Angiospermous Plants, 281
  Zirkle, Conway, Evolution, Marxian Biology and the Social Scene, 282
Breeding system in mouse-tails, 151
Briggs, Michael H., Dating the origin of life on earth, 416
Brower, Lincoln P., Speciation in butterflies of the *Papilio glaucus* group, 40, 212
Bufo arenarum, polymorphism in, 532
Buttercups, variation in, 469
Butterflies, (*Papilio glaucus*), 40, 212; (*Colias*), 64
Buzzati-Traverso, A. A. (ed.), Perspectives in Marine Biology, Review by R. G. Johnson, 149
Calvin, Melvin, Round trip from space, 362
Canalization of scute phenotype in Drosophila, 425
Canis lupus, geographical variation in, 283
Cannabis sativa, variability in, 145
Captorhinus tympanum, 99
Caspari, Ernst (with G. S. Watson), On the evolutionary importance of cytoplasmic sterility in mosquitoes, 568
Cei, J. M., Ecological and physiological observations on polymorphic populations of the toad, *Bufo arenarum* Hensel, from the Argentine, 532
Cenozoic evolution of bigtrees, 9
Chemical evolution, 362
Choristoneura hemolymph polymorphism, 537
Chromosomal aberration in *Podophyllum peltatum,* 276
Chromosomal distribution of mutator- and radiation-induced mutations in *D. melanogaster,* 526
Chromosomal evolution in Asterinae, 188
Chromosomal polymorphism in *Drosophila willistoni,* 147
Climate, area and evolution, 488
Cockerham, Clark C. (with R. C. Lewontin), The goodness-of-fit test for detecting natural selection in random mating populations, 561
Cold Spring Harbor Symposium on Quantitative Biology, vol. XXIII, (notice of book), 282; book review, 570
Colias hybrids, 64
Corrections:
  To article by W. Gajewski (Evolution, 13: 378–388), 574
  To article by Masatako Karabayashi (Evolution, 12: 286–310), 282
  To article by D. B. O. Savile (Evolution, 13: 333–343), 574
Crinia, mating calls in, 452
Cytoplasmic sterility in mosquitoes, 568
Dactylis glomerata triploids, 311

da Cunha, A. B. (with Th. Dobzhansky, O. Pavlovsky, and B. Spassky), Genetics of natural populations. XXVIII, 389
Daly, Kevin (with Harold H. Smith), Discrete populations derived by interspecific hybridization and selection in *Nicotiana,* 476
Darlington, Philip J., Jr., Area, climate and evolution, 488
Dating origin of life, 416
Delphinium gypsophilum, a species of hybrid origin, 511
Dispersal of a tropical ant fauna, 122
Dobzhansky, Th. (with A. Brito da Cunha, O. Pavlovsky and B. Spassky), Genetics of natural populations. XXVIII, 389
Drosophila antennae, 147
Drosophila melanogaster, eye pigments, 1; mutator- and radiation-induced mutations, 526; scute phenotype, 425
Drosophila simulans, eye pigments, 1
Drosophila subobscura, inversion systems, 89
Drosophila willistoni, chromosomal polymorphism in, 147
Ear, middle, evolution of, 99
Ecological barriers in evolution, 333; correction, 574
Ecological relationships of butterfly species, 212
Ecotypic variation in *Nigella,* 469
Ehrman, Lee, The antennae of Drosophila females, 147
Eocene rodents, 354
Epling, Carl (with Harlan Lewis), *Delphinium gypsophilum,* a diploid species of hybrid origin, 511
Errata:
In article by W. Gajewski (Evolution, 13: 378–388), 574
In article by Masatako Kurabayashi (Evolution, 12: 286–310), 282
In article by D. B. O. Savile (Evolution, 13: 333—343), 574
Evans, Howard P., Some comments on the evolution of Arthropoda, 147
Evolution, Marxian Biology and the Social Scene, (notice of book), 282
Evolutionary pathways, multiple, 194
Evolutionary signicance of penetration of barriers, 333; correction, 574
Exchange of Genetic Material, (book review), 570
Eye pigments in Drosophila species, 1
Fish evolution in Lake Nyasa, 440
Fish hybrids, 236
Fitness in random mating populations, 561
Forerunners of Darwin, (notice of book), 423; book review, 573
Fryer, Geoffrey, Some aspects of evolution in Lake Nyasa, 440
Gajewski, W., Evolution in the genus, *Geum,* 378; correction, 574
Gambusia hybrids, 236
Gene exchange, in *Dactylis glomerata,* 311; in the origin of *Delphinium gypsophilum,* 511; prevented in *Mimulus guttatus,* 300
Geographical barriers in evolution, 333; correction, 574
Geographical differentiation of inversion systems in *Drosophila subobscura,* 89
Geographical variation in the wolf, 283
Geum, evolution in, 378; correction, 574
Glass, Bentley, Owsei Temkin, William L. Straus, Jr. (editors), Forerunners of Darwin: 1745–1859, (notice of book), 423; review by Conway Zirkle, 573
Goldschmidt, Elisabeth (with E. Stumm-Zollinger), Geographical differentiation of inversion systems in *Drosophila subobscura,* 89
Gophers, pocket, 247
Grant, Verne, Origins of Angiospermous Plants, reviewed by, 281
Grasses, parallel development of morphology in, 418
Gymnosperms, polyploidy in, 24
Hemolymph polymorphism in a moth and the nature of sex-controlled inheritance, 537
Hemp, variability in, 145
Heslop-Harrison, J., Variability and environment, 145
Hill, W. C. Osman, (notice of book), 574
Hordeum agriocrithon, 279
Hotton III, Nicholas, The pelycosaur tympanum and early evolution of the middle ear, 99
Hubbs, Clark, Population analysis of a hybrid swarm between *Gambusia affinis* and *G. heterochir,* 236
Huziwara, Yukio, Chromosomal evolution in the subtribe Asterinae, 188
Hybrid origin of *Delphinium gypsophilum,* 511
Hybridization, in *Colias,* 64; *Drosophila,* 147; *Gambusia,* 236; *Nicotiana,* 476; *Papilio glaucus* group, 40; *Viola,* 318
Hypnotic reflex in the toad, 532
Inger, F., Reply to Sokal, 423
Interspecific hybrids, *Colias,* 64; *Drosophila,* 147; *Gambusia,* 236; *Nicotiana,* 476; *Papilio,* 40; *Viola,* 318
Interspecific mating in Drosophila, 147
Interspecific sexual behavior in butterflies, 212
Inversion systems in *Drosophila subobscura,* 89; in *Drosophila willistoni,* 389
Ives, P. T., Chromosomal distribution of mutator- and radiation-induced mutations in *D. melanogaster,* 526
Johnson, Ralph G., Perspectives in Marine Biology, reviewed by, 149
Jolicoeur, Pierre, Multivariate geographical variation in the wolf, *Canis lupus* L., 283
Jurassic ammonites, 229
Just before Darwin, (notice of book), 573
Kennerly, Thomas E., Jr., Contact between the ranges of two allopatric species of pocket gophers, 247

Khoshoo, T. N., Polyploidy in Gymnosperms, 24
Kurabayashi, Masatako, Corrections to article by (Evolution, 12: 286–310), 282
Lacustrine fauna, 440
Lepidoptera hybrids, 40, 64
Lepturus morphology, 418
Lethols in *D. melanogaster,* 526
Lewis, Harlan (with Carl Epling), *Delphinium gypsophilum,* a diploid species of hybrid origin, 511
Lewontin, R. C. (with C. Clark Cockerham), The goodness-of-fit test for detecting natural selection in random mating populations, 561
Li, C. C., Notes on relative fitness of genotypes that forms a geometric progression, 564
Life, origin of, 362, 416
Littlejohn, Murray J., Call differentiation in a complex of seven species of *Crinia* (Anura, Leptodactylidae), 452
Lorch, Jacob W., The perianth of Aristolochia —a new interpretation, 415
Mammalian characters, evolution of, 344
Mammals, origin of, 405
Manton, S. M. (with J. T. Saunders), notice of book, 574
Manual of Practical Vertebrate Morphology, A, (notice of book), 574
Mating between Drosophila species, 147
Mating calls in *Crinia,* 452
Melanesia, ponerine ants in, 122
Mezosoic mammals, 405
Millhauser, Milton, Just before Darwin, (notice of book), 573
Mimulus guttatus, gene exchange in, 300
Molecular Basis of Evolution, The, (notice of book), 574
Monerma morphology, 418
Moore, D. M., Population studies on *Viola lactea* Sm. and its wild hybrids, 318
Mosquitoes, cytoplasmic sterility in, 568
Moth (*Choristoneura*), 537
Mouse-tails, vernal pool, 151
Multiple evolutionary pathways, 194
Multivariate geographical variation in the wolf, 283
Mutations in *D. melanogaster,* 526
Mutator gene in *D. melanogaster,* 526
Nasal anatomy of reptiles, 175
National Science Foundation, announcement, 150
Natural selection, biometric tests for, 561
Newman, Lester J., Chromosomal aberrations in *Podophyllum peltatum,* 276
Nicotiana, selection following hybridization, 476
Nolte, D. J., Polygenes in eye pigment production in Drosophila species, 1
Nur, Uzi (with Daniel Zohary), Natural triploids in the orchard grass, *Dactylis glomerata* L. polyploid complex and their significance for gene flow from diploid to triploid levels, 311
Nyasa Lake, evolution in, 440
Olson, Everett C., The evolution of mammalian characters, 344
Orchard grass triploids, 311
Origins of Angiospermous Plants, (book review), 281
Origin of life, 362, 416
Padoa, Emanuele, Storia della Vita sulla Terra, (notice of book), 282
Paleoflora, evolution of psilophite, 264
Papilio glaucus group of species, 40, 212
Parsons, Thomas E., Nasal anatomy and the phylogeny of reptiles, 175
Pavlovsky, O. (with A. Brito da Cunha, Th. Dobzhansky, and B. Spassky), Genetics of natural populations. XXVIII, 389
Pelycosaur tympanum, 99
Perspectives in Marine Biology, (book review), 149
Phylogeny of mammals, 344, 405; reptiles, 175; rodents, 354
Pocket gophers, allopatric species of, 247
Podophyllum peltatum, chromosomal aberrations in, 276
Polygenic control of Drosophila eye pigments, 1
Polymorphism in *Bufo arenarum,* 532; *Choristoneura,* 537; *Drosophila subobscura,* 89; *Drosophila willistoni,* 389
Polyphyletic origin of mammals, 405
Polyploidy in Dactylis, 311; in Gymnosperms, 24
Ponerine ants, 122
Preadaptation and multiple evolutionary pathways, 194
Psilophyte paleoflora, 264
Quantitative systematics, 420, 423
Radiation-induced mutations in *D. melanogaster,* 526
Rendel, J. M., Canalization of the scute phenotype of Drosophila, 425
Reproductive isolation and mating calls in Anura, 452
Reptile nasal anatomy and phylogeny, 175
Rodent phylogeny, 354
Roe, Anne (with George Gaylord Simpson), notice of book, 574
Romer, Alfred S., The Vertebrate Story, (notice of book), 282
St. Lawrence, Patricia, Cold Spring Harbor Symposium on Quantitative Biology, vol. XXIII, reviewed by, 570
Saunders, J. T. and S. M. Manton, A Manual of Practical Vertebrate Morphology, (notice of book), 574
Savile, D. B. O., Limited penetration of barriers as a factor in evolution, 333; correction, 574
Schmidt, Karl P. Fund, announcement, 150

Scute phenotype in *Drosophila melanogaster,* 425
Selection following hybridization in *Nicotiana,* 476
Selection, natural, biometric test for, 561
Sequoia dendron species, 9
Sex-controlled inheritance in a moth, 537
Sexual behavior in butterflies, 212
Sierra Nevada bigtree forest, 9
Simpson, George Gaylord, Mesozoic mammals and the polyphyletic origin of mammals, 405; (with Anne Roe), notice of book, 574
Smith, Harold H. (with Kevin Daly), discrete populations derived by interspecific hybridization and selection in *Nicotiana,* 476
Sokal, Robert R., Comments on quantitative systematics, 420
Spassky, B. (with A. Brito da Cunha, Th. Dobzhansky and O. Pavlovsky), Genetics of natural populations. XXVIII, 389
Speciation in butterflies, 40, 212; fish, 440; tropical ants, 122
Stehr, G., Hemolymph polymorphism in a moth and the nature of sex-controlled inheritance, 537
Sterility, cytoplasmic in mosquitoes, 568
Stone, Donald E., A unique balanced breeding system in the vernal pool mouse-tails, 151
Storia della Vita sulla Terra, (notice of book), 282
Straus, W. L., Jr. (with B. Glass and O. Temkin), notice of book, 423; review of book, 573
Stumm-Zollinger, Elisabeth (with E. Goldschmidt), Geographical differentiation of inversion systems in *Drosophila subobscura,* 89
Systematics, quantitative, 420, 423
Takhtajian, A. L. (Takhtajan), Origins of Angiospermous Plants, review by Verne Grant, 281
Tateoka, Tuguo, *Lepturus* and *Monerma:* a remarkable example of parallel development of gross morphology in grasses, 418
Temkin, Owsei (with B. Glass and W. L. Straus, Jr.), notice of book, 423; review of book, 573
Toads, hypnotic reflex in, 532
Triploids in *Dactylis glomerata,* 311
Tropical ants, adaptive shift and dispersal, 122
Tympanum of pelycosaur and captorhinomorph reptiles, 99
Variability and environment, 145
Variation, geographical in the wolf, 283
Vernal pool mouse-tails, 151
Vertebrate Story, The, (notice of book), 282
Vickery, Robert K., Jr., Barriers to Gene Exchange within *Mimulus guttatus* (Scrophulariceceae), 300
Viola lactea hybrids, 318
Waisel, Yoav, Ecotypic variation in *Nigella arvensis,* L., 469
Watson, G. S. (with Ernst Caspari), On the evolutionary importance of cytoplasmic sterility in mosquitoes, 568
Wilson, Edward O., Adaptive shift and dispersal of a tropical ant fauna, 122
Wolf, geographical variation in, 283
Wood, Albert E., Eocene radiation and phylogeny of rodents, 354
Ziegler, Bernhard, Evolution in upper Jurassic ammonites, 229
Zirkle, Conway, Evolution, Marxian Biology and the Social Scene, (notice of book), 282; Forerunners of Darwin, reviewed by, 573
Zohary, Daniel, Is *Hordeum agriocrithon* the ancestor of six-rowed cultivated barley? 279; (with Uzi Nur), Natural triploids in the orchard grass, *Dactylis glomerata* L. polyploid complex and their significance for gene flow from diploid to triploid levels, 311

COLUMBIA UNIVERSITY LIBRARY
ZOOLOGY & BOTANY LIBRARY COLUMBIA UNIVERSITY

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XIII *March 1959* No. 1

CONTENTS

Polygenes in Eye Pigment Production in Drosophila Species. D. J. NOLTE 1

Late Cenozoic Evolution of the Sierran Bigtree Forest. DANIEL I. AXELROD 9

Polyploidy in Gymnosperms. T. N. KHOSHOO 24

Speciation in Butterflies of the *Papilio glaucus* Group. I. Morphological Relationships and Hybridization. LINCOLN P. BROWER .. 40

A Study of Hybrids in *Colias* (Lepidoptera, Pieridae). SHIGERU ALBERT AE 64

Geographical Differentiation of Inversion Systems in *Drosophila subobscura*. ELISABETH STUMM-ZOLLINGER and ELISABETH GOLDSCHMIDT 89

The Pelycosaur Tympanum and Early Evolution of the Middle Ear. NICHOLAS HOTTON III 99

Adaptive Shift and Dispersal of a Tropical Ant Fauna. EDWARD O. WILSON 122

Notes and Comments

Variability and Environment. J. HESLOP-HARRISON 145

The Antennae of Drosophila Females. LEE EHRMAN 147

Some Comments on the Evolution of Arthropoda. HOWARD P. EVANS 147

Perspectives in Marine Biology. RALPH G. JOHNSON 149

Announcements 150

PUBLISHED QUARTERLY

BY THE SOCIETY FOR THE STUDY OF EVOLUTION

PRINCE AND LEMON STREETS, LANCASTER, PA.

ISSUED MARCH 31, 1959

EDITORIAL BOARD

I. MICHAEL LERNER, *Editor*

ASSOCIATE EDITORS

Class of 1959

H. G. BAKER, *Berkeley*
H. L. CARSON, *St. Louis*
E. B. FORD, *Oxford*
B. PATTERSON, *Cambridge (Mass.)*
H. H. ROSS, *Urbana*
M. J. D. WHITE, *Melbourne*

Class of 1960

R. CIFERRI, *Pavia*
K. W. COOPER, *Gainesville*
O. H. FRANKEL, *Canberra*
R. F. INGER, *Chicago*
B. SCHAEFFER, *New York*
M. J. TOERRIEN, *Stellenbosch*

Class of 1961

R. B. COWLES, *Los Angeles*
A. B. DA CUNHA, *São Paulo*
R. C. ROLLINS, *Cambridge (Mass.)*
D. B. O. SAVILE, *Ottawa*
R. A. STIRTON, *Berkeley*
P. V. TERENTJEV, *Leningrad*

EVOLUTION is published quarterly by the Society for the Study of Evolution at Prince and Lemon Sts., Lancaster, Pa. Membership fee is $6.00 per year, including subscription to EVOLUTION. Subscription price to non-members: $6.00 for 1959; $7.00 thereafter.

EVOLUTION is open to papers of evolutionary interest. Manuscripts and all editorial correspondence should be addressed to the Editor at the Department of Genetics, University of California, Berkeley 4, Calif. Subscriptions are to be ordered through the office of the Treasurer, Dr. Hampton L. Carson, Dept. of Zoology, Washington University, St. Louis 5, Mo.

Numbers lost in the mail will be supplied free if written claim is received within one month of the date of issue of the next succeeding number.

Notices of change of address should be sent to the Treasurer. Directions for the mailing of reprints should be addressed to EVOLUTION, Prince and Lemon Streets, Lancaster, Pennsylvania.

Second-class postage paid at Lancaster, Pa.

Doe R.R

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XIII *June 1959* No. 2

UNIVERSITY LIBRARY JUN 9 1959

CONTENTS

A Unique Balanced Breeding System in the Vernal Pool Mouse-Tails. DONALD E. STONE 151

Nasal Anatomy and the Phylogeny of Reptiles. THOMAS S. PARSONS 175

Chromosomal Evolution in the Subtribe Asterinae. YUKIO HUZIWARA 188

Preadaptation and Multiple Evolutionary Pathways. WALTER J. BOCK 194

Speciation in Butterflies of the *Papilio glaucus* Group. II. Ecological Relationships and Interspecific Sexual Behavior. LINCOLN P. BROWER 212

Evolution in Upper Jurassic Ammonites. BERNHARD ZIEGLER 229

Population Analysis of a Hybrid Swarm between *Gambusia affinis* and *G. heterochir*. CLARK HUBBS 236

Contact between the Ranges of Two Allopatric Species of Pocket Gophers. THOMAS E. KENNERLY, JR. 247

Evolution of the Psilophyte Paleoflora. DANIEL I. AXELROD 264

Notes and Comments

Chromosomal Aberrations in *Podophyllum peltatum*. LESTER J. NEWMAN 276

Is *Hordeum agriocrithon* the Ancestor of Six-Rowed Cultivated Barley? DANIEL ZOHARY 279

"Origins of Angiospermous Plants." VERNE GRANT 281

Books Received 282

Errata 282

PUBLISHED QUARTERLY

BY THE SOCIETY FOR THE STUDY OF EVOLUTION

PRINCE AND LEMON STREETS, LANCASTER, PA.

ISSUED JUNE 5, 1959

EDITORIAL BOARD

I. MICHAEL LERNER, *Editor*

ASSOCIATE EDITORS

Class of 1959

H. G. BAKER, *Berkeley*
H. L. CARSON, *St. Louis*
E. B. FORD, *Oxford*
B. PATTERSON, *Cambridge (Mass.)*
H. H. ROSS, *Urbana*
M. J. D. WHITE, *Melbourne*

Class of 1960

R. CIFERRI, *Pavia*
K. W. COOPER, *Gainesville*
O. H. FRANKEL, *Canberra*
R. F. INGER, *Chicago*
B. SCHAEFFER, *New York*
M. J. TOERRIEN, *Stellenbosch*

Class of 1961

R. B. COWLES, *Los Angeles*
A. B. DA CUNHA, *São Paulo*
R. C. ROLLINS, *Cambridge (Mass.)*
D. B. O. SAVILE, *Ottawa*
R. A. STIRTON, *Berkeley*
P. V. TERENTJEV, *Leningrad*

EVOLUTION is published quarterly by the Society for the Study of Evolution at Prince and Lemon Sts., Lancaster, Pa. Membership fee is $6.00 per year, including subscription to EVOLUTION. Subscription price to non-members: $6.00 for 1959; $7.00 thereafter.

EVOLUTION is open to papers of evolutionary interest. Manuscripts and all editorial correspondence should be addressed to the Editor at the Department of Genetics, University of California, Berkeley 4, Calif. Subscriptions are to be ordered through the office of the Treasurer, Dr. Hampton L. Carson, Dept. of Zoology, Washington University, St. Louis 5, Mo.

Numbers lost in the mail will be supplied free if written claim is received within one month of the date of issue of the next succeeding number.

Notices of change of address should be sent to the Treasurer. Directions for the mailing of reprints should be addressed to EVOLUTION, Prince and Lemon Streets, Lancaster, Pennsylvania.

Second-class postage paid at Lancaster, Pa.

COLUMBIA UNIVERSITY
LIBRARY
SEP 4 1959

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XIII *September 1959* No. 3

CONTENTS

Multivariate Geographical Variation in the Wolf, *Canis lupus* L. PIERRE JOLICOEUR 283

Barriers to Gene Exchange within *Mimulus guttatus* (Scrophulariaceae). ROBERT K. VICKERY, JR. 300

Natural Triploids in the Orchard Grass, *Dactylis glomerata* L. Polyploid Complex and Their Significance for Gene Flow from Diploid to Triploid Levels. DANIEL ZOHARY and UZI NUR 311

Population Studies on *Viola lactea* Sm. and its Wild Hybrids. D. M. MOORE 318

Limited Penetration of Barriers as a Factor in Evolution. D. B. O. SAVILE 333

The Evolution of Mammalian Characters. EVERETT C. OLSON 344

Eocene Radiation and Phylogeny of the Rodents. ALBERT E. WOOD 354

Round Trip from Space. MELVIN CALVIN 362

Evolution in the Genus *Geum*. W. GAJEWSKI 378

Genetics of Natural Populations. XXVIII. Supplementary Data on the Chromosomal Polymorphism in *Drosophila willistoni* in its Relation to the Environment. A. BRITO DA CUNHA, TH. DOBZHANSKY, O. PAVLOVSKY and B. SPASSKY 389

Mesozoic Mammals and the Polyphyletic Origin of Mammals. GEORGE GAYLORD SIMPSON 405

Notes and Comments

The Perianth of *Aristolochia*—a New Interpretation. JACOB W. LORCH 415

Dating the Origin of Life on Earth. MICHAEL H. BRIGGS 416

Lepturus and *Monerma:* a Remarkable Example of Parallel Development of Gross Morphology in Grasses. TUGUO TATEOKA 418

Comments on Quantitative Systematics. ROBERT R. SOKAL ... 420

Reply to Sokal. ROBERT F. INGER 423

Books Received 423

PUBLISHED QUARTERLY

BY THE SOCIETY FOR THE STUDY OF EVOLUTION

PRINCE AND LEMON STREETS, LANCASTER, PA.

ISSUED SEPTEMBER 8, 1959

ZOOLOGY - BOTANY
LIBRARY
COLUMBIA UNIVERSITY

EDITORIAL BOARD

I. MICHAEL LERNER, *Editor*

ASSOCIATE EDITORS

Class of 1959

H. G. BAKER, *Berkeley*
H. L. CARSON, *St. Louis*
E. B. FORD, *Oxford*
B. PATTERSON, *Cambridge (Mass.)*
H. H. ROSS, *Urbana*
M. J. D. WHITE, *Melbourne*

Class of 1960

R. CIFERRI, *Pavia*
K. W. COOPER, *Hanover*
O. H. FRANKEL, *Canberra*
R. F. INGER, *Chicago*
B. SCHAEFFER, *New York*
M. J. TOERRIEN, *Stellenbosch*

Class of 1961

R. B. COWLES, *Los Angeles*
A. B. DA CUNHA, *São Paulo*
R. C. ROLLINS, *Cambridge (Mass.)*
D. B. O. SAVILE, *Ottawa*
R. A. STIRTON, *Berkeley*
P. V. TERENTJEV, *Leningrad*

EVOLUTION is published quarterly by the Society for the Study of Evolution at Prince and Lemon Sts., Lancaster, Pa. Membership fee is $6.00 per year, including subscription to EVOLUTION. Subscription price to non-members: $6.00 for 1959; $7.00 thereafter.

EVOLUTION is open to papers of evolutionary interest. Manuscripts and all editorial correspondence should be addressed to the Editor at the Department of Genetics, University of California, Berkeley 4, Calif. Subscriptions are to be ordered through the office of the Treasurer, Dr. Hampton L. Carson, Dept. of Zoology, Washington University, St. Louis 5, Mo.

Numbers lost in the mail will be supplied free if written claim is received within one month of the date of issue of the next succeeding number.

Notices of change of address should be sent to the Treasurer. Directions for the mailing of reprints should be addressed to EVOLUTION, Prince and Lemon Streets, Lancaster, Pennsylvania.

Second-class postage paid at Lancaster, Pa.

COLUMBIA UNIVERSITY
LIBRARY
NOV 13 1960

EVOLUTION

INTERNATIONAL JOURNAL OF ORGANIC EVOLUTION

Vol. XIII *December 1959* No. 4

CONTENTS

Canalization of the Scute Phenotype of Drosophila. J. M. RENDEL 425

Some Aspects of Evolution in Lake Nyasa. GEOFFREY FRYER 440

Call Differentiation in a Complex of Seven Species of *Crinia* (Anura, Leptodactylidae). MURRAY J. LITTLEJOHN 452

Ecotypic Variation in *Nigella arvensis* L. YOAV WAISEL 469

Discrete Populations Derived by Interspecific Hybridization and Selection in *Nicotiana*. HAROLD H. SMITH and KEVIN DALY 476

Area, Climate and Evolution. PHILIP J. DARLINGTON, JR. 488

Delphinium gypsophilum, a Diploid Species of Hybrid Origin. HARLAN LEWIS and CARL EPLING 511

Chromosomal Distribution of Mutator- and Radiation-induced Mutations in *D. melanogaster*. P. T. IVES 526

Ecological and Physiological Observations on Polymorphic Populations of the Toad, *Bufo arenarum* Hensel, from Argentine. J. M. CEI 532

Hemolymph Polymorphism in a Moth and the Nature of Sex-controlled Inheritance. G. STEHR 537

Notes and Comments

The Goodness-of-fit Test for Detecting Natural Selection in Random Mating Populations. R. C. LEWONTIN and C. CLARK COCKERHAM 561

Notes on Relative Fitness of Genotypes that Forms a Geometric Progression. C. C. LI 564

On the Evolutionary Importance of Cytoplasmic Sterility in Mosquitoes. ERNST CASPARI and G. S. WATSON 568

Exchange of Genetic Material. PATRICIA ST. LAWRENCE 570

Forerunners of Darwin. CONWAY ZIRKLE 573

Books Received 573

Errata 574

Index for Volume XIII

PUBLISHED QUARTERLY

BY THE SOCIETY FOR THE STUDY OF EVOLUTION

PRINCE AND LEMON STREETS, LANCASTER, PA.

ISSUED DECEMBER 30, 1959

EDITORIAL BOARD

I. MICHAEL LERNER, *Editor*

ASSOCIATE EDITORS

Class of 1959

H. G. BAKER, *Berkeley*
H. L. CARSON, *St. Louis*
E. B. FORD, *Oxford*
B. PATTERSON, *Cambridge (Mass.)*
H. H. ROSS, *Urbana*
M. J. D. WHITE, *Melbourne*

Class of 1960

R. CIFERRI, *Pavia*
K. W. COOPER, *Hanover*
O. H. FRANKEL, *Canberra*
R. F. INGER, *Chicago*
B. SCHAEFFER, *New York*
M. J. TOERRIEN, *Stellenbosch*

Class of 1961

R. B. COWLES, *Los Angeles*
A. B. DA CUNHA, *São Paulo*
R. C. ROLLINS, *Cambridge (Mass.)*
D. B. O. SAVILE, *Ottawa*
R. A. STIRTON, *Berkeley*
P. V. TERENTJEV, *Leningrad*

EVOLUTION is published quarterly by the Society for the Study of Evolution at Prince and Lemon Sts., Lancaster, Pa. Membership fee is $6.00 per year, including subscription to EVOLUTION. Subscription price to non-members: $6.00 for 1959; $7.00 thereafter.

EVOLUTION is open to papers of evolutionary interest. Manuscripts and all editorial correspondence should be addressed to the Editor at the Department of Genetics, University of California, Berkeley 4, Calif. Subscriptions are to be ordered through the office of the Treasurer, Dr. Hampton L. Carson, Dept. of Zoology, Washington University, St. Louis 5, Mo.

Numbers lost in the mail will be supplied free if written claim is received within one month of the date of issue of the next succeeding number.

Notices of change of address should be sent to the Treasurer. Directions for the mailing of reprints should be addressed to EVOLUTION, Prince and Lemon Streets, Lancaster, Pennsylvania.

Second-class postage paid at Lancaster, Pa.

INFORMATION FOR CONTRIBUTORS

Contributions to EVOLUTION may be in the fields of genetics, cytology, plant and animal taxonomy, paleontology, ecology, anthropology, bacteriology, or any other field of biology, provided that the facts and conclusions presented are of primary interest to the student of evolution. Descriptive material shall be included only so far as it is the basis of the evolutionary conclusions. Manuscripts are examined for suitability by members of the Editorial Board or other competent critics.

The length of manuscripts will be limited to twenty printed pages unless an exception is granted by the Editorial Board. Short manuscripts and discussions will be included in a separate section, "Notes and Comments." Half tones and tabular material will have to be kept within economic limits.

Proof should be corrected immediately on receipt, and returned at once with manuscript to the Editor. Authors should leave forwarding directions whenever they move away from the address sent with the manuscript. Ordinarily page proof will not be sent to the author. Costs of changes in proof from the original copy will be charged to the author.

Reprints should be ordered when galley proof is returned. There is usually a delay of several weeks after publication before the author receives the reprints ordered. Illustrations and cuts will be destroyed, unless their return is requested on the reprint order.

Manuscripts should be typewritten in double spacing with ample margins and on only one side of the paper. The Editor should have *one carbon copy in addition* to the original manuscript. The author should keep one carbon copy.

Tables and captions for figures should be typed on separate sheets from the text. The legends for several figures may be placed on a single sheet.

Illustrations, including maps, should be referred to as "figures" wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures. All figures and plates are reduced to a maximum of 4⅞ inches in width and 6½ inches in length. Reductions may be stated in the caption but not on the figure.

All references to literature should be collected into a list of "Literature Cited" at the end of the article. See recent numbers of EVOLUTION for accepted style.

Titles should be short, preferably less than seven words. Footnote material should be incorporated in the text whenever possible.

Every article of more than a few pages should have a summary limited to less than four per cent of the length of the paper; it is to be complete in itself, and understandable without reference to the body of the article. The scope of the paper should be stated in an introductory paragraph.

Authors are requested to prepare abstracts of their papers to be returned with the proof to the Editor to be forwarded to Biological Abstracts.

Manuscripts should be addressed to

I. Michael Lerner
Department of Genetics
University of California
Berkeley 4, Calif.

THE SOCIETY FOR THE STUDY OF EVOLUTION

Officers for 1959

President: EDGAR ANDERSON, Missouri Botanical Garden, St. Louis, Mo.

Vice Presidents: J. W. GREGOR, Pontlandfield, Roslin, Midlothian, Scotland
HARLAN LEWIS, University of California, Los Angeles, Calif.
N. TINBERGEN, University Museum, Oxford, England

Secretary (1959–1961): HERBERT H. ROSS, State Natural History Survey, Urbana, Ill.

Treasurer (1958–1960): HAMPTON L. CARSON, Washington University, St. Louis, Mo.

Editor (1959–1961): I. MICHAEL LERNER, University of California, Berkeley, Calif.

Elected Members of the Council:

Class of 1959: CHARLES M. BOGERT (New York), CARL EPLING (Los Angeles)
Class of 1960: BOBB SCHAEFFER (New York), WILLIAM C. STEERE (New York)
Class of 1961: W. FRANK BLAIR (Austin), JENS CLAUSEN (Stanford)

OBJECTS: The object of the Society for the Study of Evolution, which was founded in March, 1946, is the promotion of the study of organic evolution and the integration of the various fields of science concerned with evolution. The Society endeavors to accomplish this through the publication of the journal and through meetings and working committees.

MEMBERSHIP: Membership is open to all those who are interested in the study of evolution. Members are elected by the Council. There are three classes of members:

Members—Dues $6.00 (including subscription to EVOLUTION)
Sustaining Members—Dues $12.00
Patrons—Single fee $1,000.

All members in good standing receive EVOLUTION.

Applications for membership should be directed to the Secretary, to whom all correspondence regarding membership should be addressed. Institutions may not become members but may subscribe to EVOLUTION. All checks should be made payable to The Society for the Study of Evolution.

3887
TM